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Full Length Article

Development and validation of an *in vitro* model to study thrombin generation on the surface of catheters in platelet-poor and platelet-rich plasma

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ABSTRACT

Introduction: Coagulation activation on medical devices remains a significant problem as it can lead to dramatic thromboembolic complications. Understanding its poorly described mechanisms and finding optimal pharmacological prevention means is crucial to improve patient safety.
Methods: We developed an *in vitro* model to study thrombin generation (TG) initiated by the contact of plasma with the surface of catheters. Interventional cardiology catheters were cut into segments and inserted in the bottom of multi-well plates; TG was then measured with the calibrated automated thrombogram (CAT). Model performance (analytical, intra- and inter-individual variability) was investigated and compared with activation of thrombin generation by tissue factor (TF) or contact pathway activator (ellagic acid), in the presence (PRP) and absence (PPP) of platelets. Model response to unfractionated heparin (UFH) was also assessed.
Results: TG was greater when measured in presence of catheter segments, compared to conditions without activators. The analytical variability of the model was good (CV $\leq$ 5 %), both with PPP and PRP. Intra-individual variability was between 15 and 30 % with PPP and between 10 and 15 % with PRP. Inter-individual variability was between 15 and 30 % with both kinds of plasma samples. The analytical performance of the catheter-initiated TG model was equivalent to that observed when TG was initiated with TF or ellagic acid. Catheter-initiated TG was measurable until 0.1 IU/mL UFH with PPP and until 1.0 IU/mL UFH with PRP, highlighting the crucial requirement of platelets.
Conclusion: Our model is suitable for studying TG initiated with catheters. Inhibition of TG by UFH is overestimated in the absence of platelets.

1. Introduction

The use of medical devices to support cardiocirculatory functions, such as extracorporeal circulation devices (cardiopulmonary bypass (CPB), extracorporeal oxygenation (ECMO)), left ventricular assistance devices (LVAD), vascular stents, mechanical heart valves (MHV) or interventional cardiology catheters, has been growing substantially over the last decades. The contact of blood with these artificial surfaces results in the activation of hemostasis and increases the risk of thrombosis [1]. In clinical practice, this can result in serious complications for the

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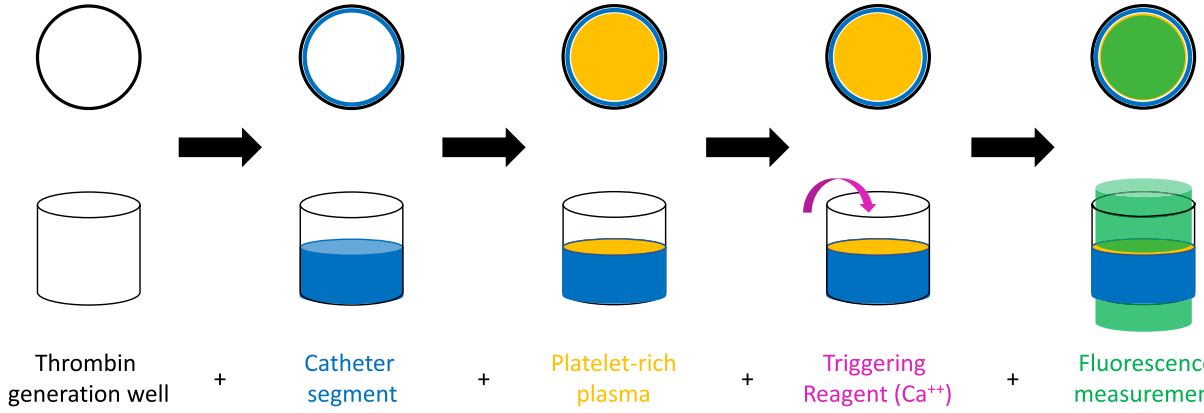

Fig. 1. Experimental model of *in vitro* catheter-initiated thrombin generation. The lower part of the figure represents a lateral view of the thrombin generation well and the upper part of the figure a view from above. An interventional cardiology catheter is flattened, cut into a rectangular segment, and then inserted into the bottom of a microplate well to cover the entire circumference of the well. PRP is added with the triggering reagent, containing calcium ions and the fluorogenic substrate, into the well. The position of the catheter segment does not interfere with the fluorogenic measurement of the thrombogram, which reads the fluorescence in the center of the well from bottom to top.

patient, such as strokes in patients with LVAD and MHV or related to interventional cardiology catheters [2–6], and/or the dysfunction of the used equipment (occlusion of ECMO oxygenators or dialytic fibers, or prosthetic valve thrombosis [7–9]).

The exact mechanisms of the activation of hemostasis on artificial surfaces are still poorly understood. *In vitro* mechanistic studies have mainly been conducted using platelet-poor plasma (PPP), focusing on factor XII auto-activation on negatively charged surfaces [10–18]. However, pre-clinical data suggest that platelets may play a role in thrombogenesis by interacting among others with adsorbed fibrinogen on the surface through integrin αIIbβ3 [19–22]. The precise role of platelets in thrombin generation initiated by these surfaces is therefore potentially critical and to the best of our knowledge has never been studied so far, which represents a major gap.

To mitigate this thrombotic risk, patients receive anticoagulants or antiplatelet agents for the duration of the device use – sometimes a lifetime, which exposes them to bleeding complications [23]. The choice of antithrombotic treatment used, often heparin in the acute phase, and the dosages used are often based on old studies of poor quality, or on clinical well-established use. Few data from well-designed studies are available to compare the relative effectiveness of different classes of anticoagulants in mitigating surface-initiated thrombin generation on medical devices [23]. The limited studies available have been performed in the absence of platelets [14,18] while their role in thrombin generation is important by acting as a complex surface for binding coagulation factors and by releasing coagulation factors (II, V…), activating molecules (tissue factor (TF), polyphosphates…), coagulation inhibitors (tissue factor pathway inhibitor…) and procoagulant extracellular vesicles [24]. In addition, *in vivo* platelet activation results in the release of platelet factor 4 (PF4) which neutralizes some of the heparin present [25,26]; working in the absence of platelets therefore results in a significant overestimation of the effect of heparin [27,28]. These uncertainties prevent optimal management of the thrombotic risk associated with medical devices, increasing the risk of complications [29].

This work aimed to develop an experimental model of thrombin generation in the presence of platelets to study the activation of hemostasis on the surface of catheters, and its pharmacological prevention.

2. Materials and methods

An experimental *in vitro* model was developed to measure thrombin generation initiated by catheter segments in the presence of platelets. The performance of the model was evaluated in terms of repeatability, reproducibility, and inter-individual variation, and compared with thrombin generated in response to tissue factor or ellagic acid provided by standard commercial reagents. The response of the model to unfractionated heparin (UFH) has also been evaluated.

2.1. Catheter-initiated thrombin generation model

The model uses interventional cardiology catheters (Launcher®, Medtronic, Minneapolis, USA; outer diameter 1.77 mm) to initiate thrombin generation measured in plasma using the Calibrated Automated Thrombogram (CAT, Diagnostica Stago, Asnières-sur-Seine, France). This catheter was chosen because it activated coagulation slightly faster than the other catheters tested (Fig. S1).

Interventional cardiology catheters were flattened, cut into identical 1.9 mm rectangular segments, and inserted into the bottom of the wells of a flat-bottom microplate (Immulon® 2 HB flat bottom microtiter plates, Thermofisher, Rochester, USA) in such a way as to cover the entire lower part of the well circumference without obstructing the bottom through which the fluorescence is read (Fig. 1).

Thrombin generation was measured with the CAT system as previously described [30]. Briefly, 80 μL of citrated plasma (PPP or platelet-rich plasma (PRP)) was added to each well of a 96-well flat-bottom microplate with 20 μL of initiator reagent (PPP low reagent (1 pM TF and 4 μM phospholipid vesicles; Diagnostica Stago), PRP reagent (1 pM TF without phospholipid vesicles; Diagnostica Stago), Dade Actin FS (100 μM ellagic acid and soy phosphatides; Siemens Healthcare Diagnostics, Erlangen, Germany), or phosphate-buffered saline (PBS)). After 10 min of incubation and brief automated stirring in a Fluoroskan FL (Thermo Scientific, Illkirch, France), 20 μL of trigger reagent (FluCa reagent, Stago) was added to each well by the instrument. Three calibrator wells per plasma (PPP and PRP) were also prepared on each plate for each volunteer: 80 μL of plasma, 20 μL of calibrator (Thrombin Calibrator, Diagnostica Stago), and 20 μL of trigger reagent. Fluorescence signals were read and recorded using a Fluoroskan FL fluorometer. All tests and calibrations were performed in triplicate. Within a triplicate, if one of the three thrombin generation curves was aberrant, it was removed. As the model will always be used in triplicates, the results will only show the mean of the triplicate.

2.2. Experimental design

Blood samples were taken from healthy volunteers after approval by the ethics committee of the CHU UCL NAMUR (NUB: B03920096633) and after providing written informed consent; the study complies with

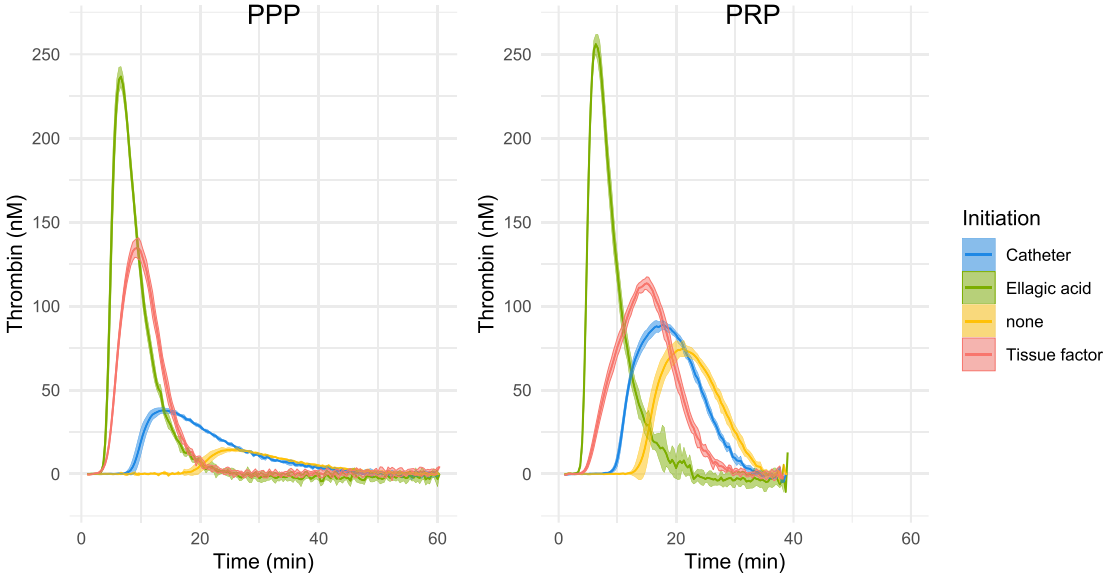

Fig. 2. Analytical variability. Thrombin generation in presence of catheters, ellagic acid and tissue factor, compared to thrombin generation in absence of any activator (but plastic of the microwells). Thrombin generation was studied with PPP (left panel) or PRP (right panel) in presence of catheter segments (blue curves), ellagic acid (Dade Actin FS diluted 1/100; green curves), tissue factor (PPP low reagent for PPP or PRP reagent for PRP; red curves) or no added reagent (yellow curves). Experiments were performed six times in triplicates on the same plate and using the same healthy volunteer's plasma; the line represents the mean of the six replicates and the ribbon represents one standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the Declaration of Helsinki. Subjects had to be <35 years old, not taking any medication and not having any systemic disorder. Peripheral venous blood was collected from fasting subjects into 2.7 mL 109 mM citrate tubes (BD, Erembodegem, Belgium) after collection of a discard tube. The tubes were allowed to stand for 15 min before centrifugation: 180*g* for 10 min at room temperature to obtain PRP and double centrifugation at 1500 *g* for 15 min at room temperature to obtain PPP.

Thrombin generation was measured as described above. Four initiating conditions were studied: (i) spontaneous initiation in the well (addition of 20 μL of PBS in place of the activator reagent), (ii) initiation in contact with a catheter segment (addition of 20 μL of PBS in place of the activator reagent), (iii) initiation with tissue factor (PPP low reagent for PPP wells and PRP reagent for PRP wells), and (iv) initiation with an intrinsic pathway initiator (ellagic acid; Dade Actin FS (Siemens Healthcare Diagnostics) diluted 100-fold to achieve a final concentration of 1 μM). Ellagic acid was chosen rather than silica or kaolin, which are particulate activators that could be less suitable for the measurement of thrombin generation [31].

The repeatability of the model was assessed by measuring thrombin generation six times in triplicates under each of four initiating conditions (no activator, catheter, tissue factor, or ellagic acid) on the same plate using plasma from the same healthy volunteer.

The reproducibility of the model was assessed by measuring thrombin generation in triplicate under the four initiating conditions in plasma from the same healthy volunteer on six different days over six months.

The interindividual variability of the model was assessed by measuring thrombin generation in the four initiating conditions in six healthy volunteers – three non-menopausal women not taking estro-progestative pills or hormone therapy – and three men.

To test how coagulation is initiated in our catheter model, we measured thrombin generation initiated by a catheter segment in the presence of an inhibitory anti-TF antibody or corn trypsin inhibitor (CTI). PPP or PRP was incubated with an inhibitory anti-TF antibody (clone SBTF-1, BioCytex, Marseille, France) at the previously validated concentration of 10 μg/mL for 30 min [32]. Similarly, CTI (Prolytix, Essex Junction, Vermont, United States) was added to PPP or PRP at a concentration of 100 μg/mL and an incubation period of 30 min was also observed; this concentration was chosen because it doubled the catheter-initiated clotting time in an experimental model using PPP [12]. The plasma was then transferred into the reaction well containing the catheter segment and 20 μL of PBS in place of the initiating reagent and thrombin generation was measured with the CAT system as previously described.

As an exploratory analysis to illustrate the need to work in the presence of platelets to assess the effect of UFH, the response of the model to UFH was also evaluated, and compared between PPP and PRP. UFH (Heparin Leo, Leo Pharma, Belgium; 0, 0.1, 0.3, 0.5, 0.7, 1.0 IU/mL, final concentrations) was added to plasma from two healthy volunteers. Thrombin generation initiated in contact with catheter segments was then measured as previously described.

3.3. Statistical methods

Results are presented as mean (+/− standard deviation (SD)). Thrombin generation lag time (LT), peak height (PH) and endogenous thrombin potential (ETP) were measured using the dedicated software (Thrombinoscope, Thrombinoscope BV, Maastricht, Netherlands). Correlation was assessed using Spearman's rank correlation method. Comparisons between thrombin generation without activator and that initiated by each of the three activators (tissue factor, ellagic acid, catheters) were performed using paired Student's *t*-tests. Alpha was set at 0.05. Analysis and figures were performed in R (version 4.2.3).

3. Results

3.1. Thrombin generation initiated in contact with catheter segments

Thrombin generation was increased in the presence of catheter segments, compared with no catheter (Fig. 2) with both PPP and PRP: LT was decreased by 10.4 (−54 %) and 4.8 min (−32 %), PH increased by 23.8 (+169 %) and 13.9 (+19 %) nM and ETP by 383.4 (+161 %) and 281.9 (+31 %) nM*min in PPP and PRP, respectively ($p < 0.001$ for all).

With PRP, a good correlation (Spearmans' $r = 0.94$, $p = 0.02$) was

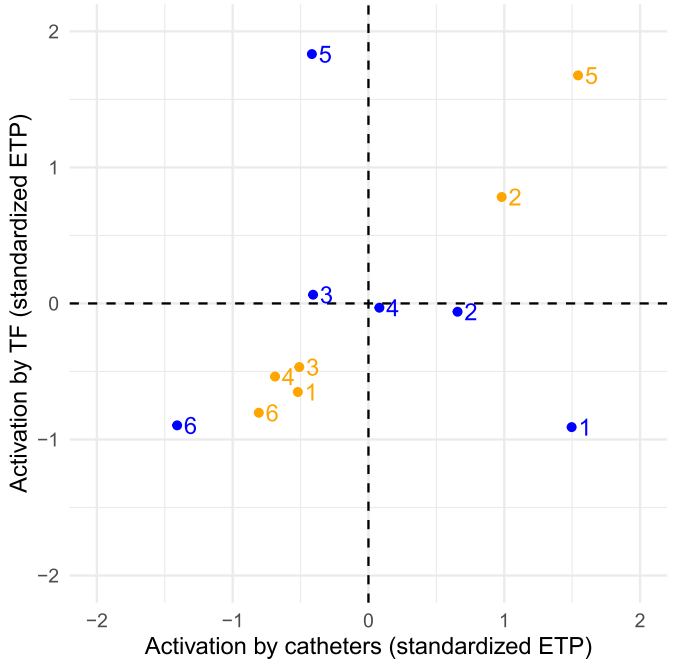

Fig. 3. Thrombin generated in response to the addition of tissue factor (y-axis) or the presence of catheter segments (x-axis). Each volunteer included, numbered from 1 to 6, is represented by two dots: one for measurements performed with PPP (blue dots), one for measurements performed with PRP (orange dots). Endogenous thrombin potential (ETP) values measured in response to catheter and tissue factor initiation were standardised for each individual to facilitate comparison between the two initiating conditions. Standardisation was achieved by subtracting the mean and dividing by the standard deviation of the ETP values for all individuals: zero is the average of the group and a value above zero indicates an ETP above the group average. Each point represents the triplicate mean of a healthy volunteer. Points in the lower left and upper right quadrants indicate a good correlation between tissue factor and catheter response. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Analytical variability of the model with coefficients of variation (%).

Plasma	Activator	LT (s)	ETP (nM*min)	PH (nM)
PPP	None	19.1 ± 1.2	247 ± 28	14.6 ± 1.3
		6.0 %	11.4 %	9.1 %
	Catheter	**8.9 ± 0.5**	**624 ± 28**	**37.9 ± 1.8**
		5.7 %	**4.5 %**	**4.6 %**
	Tissue factor	4.7 ± 0.04	1096 ± 57	135.1 ± 5.5
		1.0 %	5.2 %	4.1 %
	Ellagic acid	4.2 ± 0.2	1428 ± 81	237.4 ± 6.1
		3.6 %	5.7 %	2.6 %
PRP	None	14.6 ± 0.8	925 ± 71	74.8 ± 4.5
		5.5 %	7.7 %	6.0 %
	Catheter	**10.1 ± 0.2**	**1180 ± 17**	**89.0 ± 3.3**
		2.3 %	**1.4 %**	**3.7 %**
	Tissue factor	5.8 ± 0.2	1364 ± 44	113.5 ± 3.4
		4.1 %	3.2 %	3.0 %
	Ellagic acid	4.1 ± 0.1	1512 ± 121	259.1 ± 7.1
		2.2 %	8.0 %	2.7 %

Mean ± standard deviation and coefficient of variation (%) of the six measurements performed in triplicate.
PPP, platelet-poor plasma; PRP, platelet-rich plasma; LT, lag time; ETP, endogenous thrombin potential; PH, peak height.

observed between the amount of thrombin generated in response to tissue factor initiation and that generated in response to catheters (contact phase). On the other hand, in PPP, some individuals generated more thrombin in response to tissue factor than to catheters (*e.g.* volunteer no. 5; Fig. 3) or conversely in response to catheters than to tissue factor (*e.g.* volunteer no. 1; Fig. 3).

Table 2
Intraindividual variability with coefficients of variation (%).

Plasma	Activator	LT (s)	ETP (nM*min)	PH (nM)
PPP	none	35.0 ± 10.1	103 ± 76	7.8 ± 4.3
		29.0 %	73.2 %	55.3 %
	Catheter	**15.2 ± 3.5**	**392 ± 134**	**27.2 ± 7.4**
		22.6 %	**34.2 %**	**27.1 %**
	Tissue factor	4.3 ± 0.6	1216 ± 125	155.9 ± 15.8
		13.6 %	10.3 %	10.1 %
	Ellagic acid	6.3 ± 1.5	1321 ± 180	189.7 ± 34.8
		24.3 %	13.7 %	18.4 %
PRP	none	15.9 ± 2.4	1160 ± 151	84.8 ± 9.6
		14.8 %	13.0 %	11.3 %
	Catheter	**9.8 ± 1.1**	**1369 ± 187**	**103.6 ± 10.8**
		10.9 %	**13.7 %**	**10.4 %**
	Tissue factor	5.5 ± 0.5	1486 ± 212	115.6 ± 14.3
		8.7 %	14.2 %	12.4 %
	Ellagic acid	5.2 ± 0.9	1740 ± 234	235.7 ± 30.7
		16.6 %	13.4 %	13.0 %

Mean ± standard deviation and coefficient of variation (%) of the six measurements performed with the plasma of the same healthy volunteer collected and analyzed on six different days over six months. Each measure was performed in triplicate.
PPP, platelet-poor plasma; PRP, platelet-rich plasma; LT, lag time; ETP, endogenous thrombin potential; PH, peak height.

Table 3
Interindividual variability with coefficients of variation (%).

Plasma	Activator	LT (s)	ETP (nM*min)	PH (nM)
PPP	none	26.7 ± 5.5	294 ± 130	24.0 ± 8.7
		20.7 %	44.2 %	36.1 %
	Catheter	**13.1 ± 2.4**	**598 ± 153**	**41.4 ± 9.5**
		18.6 %	**25.7 %**	**23.0 %**
	Tissue factor	4.6 ± 0.7	1305 ± 323	209.0 ± 76.8
		14.2 %	24.8 %	36.7 %
	Ellagic acid	5.9 ± 1.3	1411 ± 252	208.3 ± 80.6
		21.6 %	17.9 %	38.7 %
PRP	none	13.2 ± 2.4	1305 ± 332	115.3 ± 31.8
		18.3 %	25.4 %	27.6 %
	Catheter	**8.5 ± 1.4**	**1569 ± 372**	**140.1 ± 38.8**
		16.5 %	**23.7 %**	**27.7 %**
	Tissue factor	5.5 ± 0.6	1678 ± 448	159.3 ± 50.3
		11.3 %	26.7 %	31.6 %
	Ellagic acid	4.8 ± 0.7	1797 ± 419	258.9 ± 32.8
		13.9 %	23.3 %	12.7 %

Mean ± standard deviation and coefficient of variation (%) of the six healthy volunteers' results (each performed in triplicate). For each healthy volunteer, thrombin generation was measured on a different day on a different plate.
PPP, platelet-poor plasma; PRP, platelet-rich plasma; LT, lag time; ETP, endogenous thrombin potential; PH, peak height.

3.2. Analytical performance of the model

The catheter model displayed a good reproducibility both in PPP and PRP, with coefficients of variation of 5 % or less for the main TG parameters. The variability was comparable to those of TG initiated with tissue factor or ellagic acid (Table 1 and Table S1).

Intraindividual variability was assessed over six months and was calculated between 20 and 35 % in PPP and between 10 and 15 % in PRP for LT, ETP and PH (Table 2 and Table S2).

To measure interindividual variability, we recruited six healthy volunteers (three males and three females) with a median age of 27 (min-max: 24–32). Median platelet count in PRP samples was 329 G/L (min-max: 296–426 10^9/L). Interindividual variability was high both for PPP and PRP (between 15 and 30 %), but comparable to those observed in response to tissue factor or ellagic acid (Table 3 and Table S3).

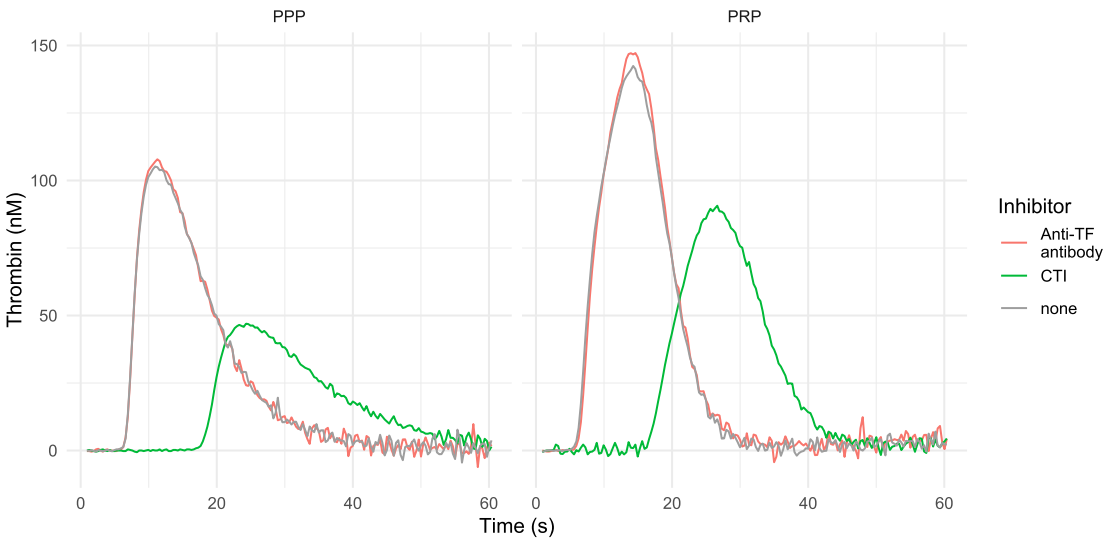

Fig. 4. Thrombin generation (TG) initiated by catheter segments in the presence of an inhibitory anti-TF antibody or corn trypsin inhibitor (CTI). An inhibitory anti-TF antibody (clone SBTF-1, Biocytex; 10 μg/mL) or CTI (Prolytix; 100 μg/mL) was incubated with PPP (left panel) or PRP (right panel) for 30 min before being added to the wells. Thrombin generation was measured in triplicate as described in the Methods. The anti-TF antibody had no effect on TG, while CTI considerably prolonged the lag time and reduced the amount of thrombin generated.

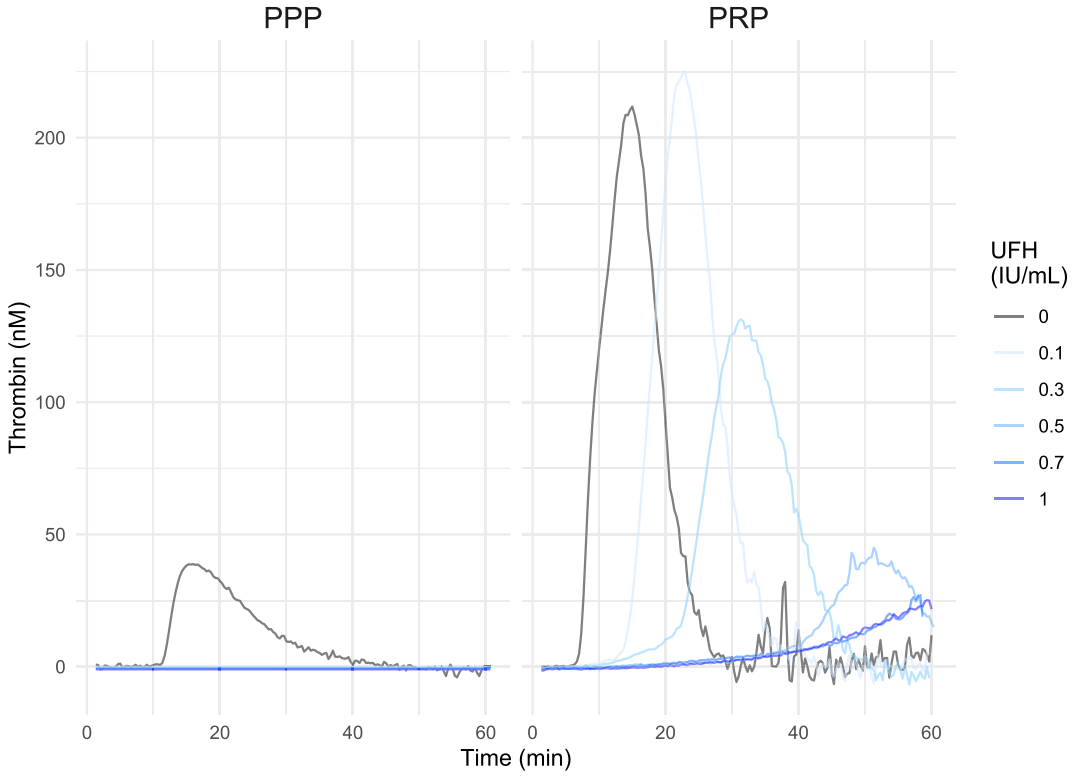

Fig. 5. Catheter model response to unfractionated heparin (UFH). Thrombin generation was studied with PPP (left panel) or PRP (right panel) in presence of increasing concentrations of UFH (up to 1 IU/mL) at the contact of catheter segments. The figure represents the mean of the triplicate. With PPP, UFH concentration of 0.1 IU/mL was able to suppress thrombin generation whereas with PRP, thrombin was detectable up to 1 IU/mL.

3.3. Initiation of coagulation in the model

In PRP, the addition of CTI 100 μg/mL led to a significant prolongation of the LT (17.51 min with CTI *vs.* 6.71 min without; Fig. 4). In contrast, the addition of an anti-TF antibody at 10 μg/mL was not associated with an increase in thrombin generation LT (6.76 min with SBTF-1 *vs.* 6.71 min without).

In PPP, we also observed a prolongation of the LT with CTI (18.12 min with CTI *vs.* 7.04 min without), but the anti-TF antibody had no effect on thrombin generation LT (7.10 min with SBTF-1 SBTF-1 *vs* 7.04 min without).

3.4. Model response to UFH

In the absence of platelets, thrombin generation was completely suppressed from 0.1 IU/mL for the first healthy volunteer (Fig. 5) and

from 0.3 IU/mL for the second (not shown). In sharp contrast, when studied in PRP, thrombin generation remained measurable up to 0.7 IU/mL for the first volunteer and 1.0 IU/mL for the second.

4. Discussion

Our *in vitro* thrombin generation model offers a reliable and reproducible method to study contact activation initiated by catheter segments in PRP. With analytical variability of <5 %, intra-individual variability of <15 %, and inter-individual variability of <30 % for the main parameters of thrombin generation (LT, PH, ETP) in PRP, its performance is equivalent to what is expected from the literature when TG is initiated with weak triggering reagents (*e.g.*, PPP low reagent) [33–37]. For example, Rudez G et al. reported inter-individual CVs of 23.8 %, 34.4 % and 21.3 % and intra-individual CVs of 16.2 %, 30.7 % and 10.6 % for thrombin generation ETP, PH and LT, respectively, measured with PPP and TF initiation on 40 healthy volunteers sampled 13 times over a one-year period [33].

We observed similar overall variability when thrombin generation was measured after activation by the tissue factor pathway (PPP low/PRP reagent), compared with the contact pathway (catheter or ellagic acid). Interestingly, the response to the contact pathway correlated well with the tissue factor response in the presence of platelets, which was less the case when measured in platelet-poor plasma. Although it is difficult to explain this observation, as the interactions between the different components of hemostasis are very complex, it reinforces our hypothesis of using a model that is as integrative as possible. We were also able to demonstrate that coagulation activation in our catheter model was indeed dependent on the contact system and not on TF.

Several models have already been used in the literature to study thrombin generation on catheter surfaces [14,17,18]. However, all of them used PPP, where platelets probably play a crucial role in contact initiation and the effect of UFH, as discussed earlier. Furthermore, the analytical performance of these models has not been published, whereas our results show high variability when measured in the absence of platelets, probably because thrombin generation is then very low, and therefore more variable. Other models such as the Badimon chamber or the Chandler's loop have also been described, but all these investigate thrombogenicity of blood rather than of foreign bodies (in this case, a catheter) and assess hemostasis less finely than thrombin generation assays [38,39].

In our model, inhibition of thrombin generation by UFH was greater when measured in PPP, compared with PRP. *In vivo*, activated platelets release the content of their granules, including platelet factor 4 (PF4, or CXCL4), which binds with high affinity to heparin, preventing its interaction with antithrombin [40], which could explain at least in part the difference in inhibition of thrombin generation by UFH between PPP and PRP. Measurement of inhibition of thrombin generation by UFH is therefore overestimated in the absence of functional, activated platelets. Similar results had also been identified using thrombin generation initiated with calcium ionophore or dilute tissue thromboplastin [27,28,41]. Assessing the effect of UFH or comparing it with other anticoagulants therefore requires working in the presence of functional platelets.

This model is incomplete, however, as it does not consider the potential contribution of erythrocytes and leukocytes to thrombin generation, nor does it allow the structure of the fibrin network to be assessed [42,43]. Moreover, hemostasis is assessed under static conditions, in the absence of flow [44,45]. However, this model has the advantage of being highly reproducible, and of being able to accurately measure the activation kinetics of coagulation factors. It will therefore enable us to take our understanding of contact activation one step further.

This model will enable us to study the mechanisms of coagulation activation on catheter surfaces, leading to thrombin generation and, ultimately, clot formation. The use of different coagulation inhibitors and platelets will allow the initiation of thrombin generation to be dissected and could contribute to improving the biocompatibility of medical devices. This model will also enable us to study the various possibilities for pharmacological inhibition of thrombin generation at the interface between blood and foreign surfaces, thereby providing a better understanding of the place of the different pharmacological inhibitors available and helping to optimize patient management. All in all, this model will contribute to a better understanding of contact activation on the surface of medical devices and optimize patient care.

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CRediT authorship contribution statement

M. Hardy: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **J. Douxfils:** Writing – review & editing, Methodology, Conceptualization. **O. Xhaet:** Writing – review & editing. **B. Robaye:** Writing – review & editing. **S. Lessire:** Writing – review & editing. **T. Lecompte:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **F. Mullier:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michael hardy reports financial support was provided by Fund for Scientific Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of interest

MH, BR, OX, SL, TL: none.

JD is the CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter, is a coinventor of the DP-Filter (patent application number: PCT/ET2019/052903) and reports personal fees from Daiichi-Sankyo, Mithra Pharmaceuticals, Diagnostica Stago, Roche and Roche Diagnostics, outside the submitted work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.109194.

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