CONGRÈS FRANÇAIS D'HÉMOSTASE

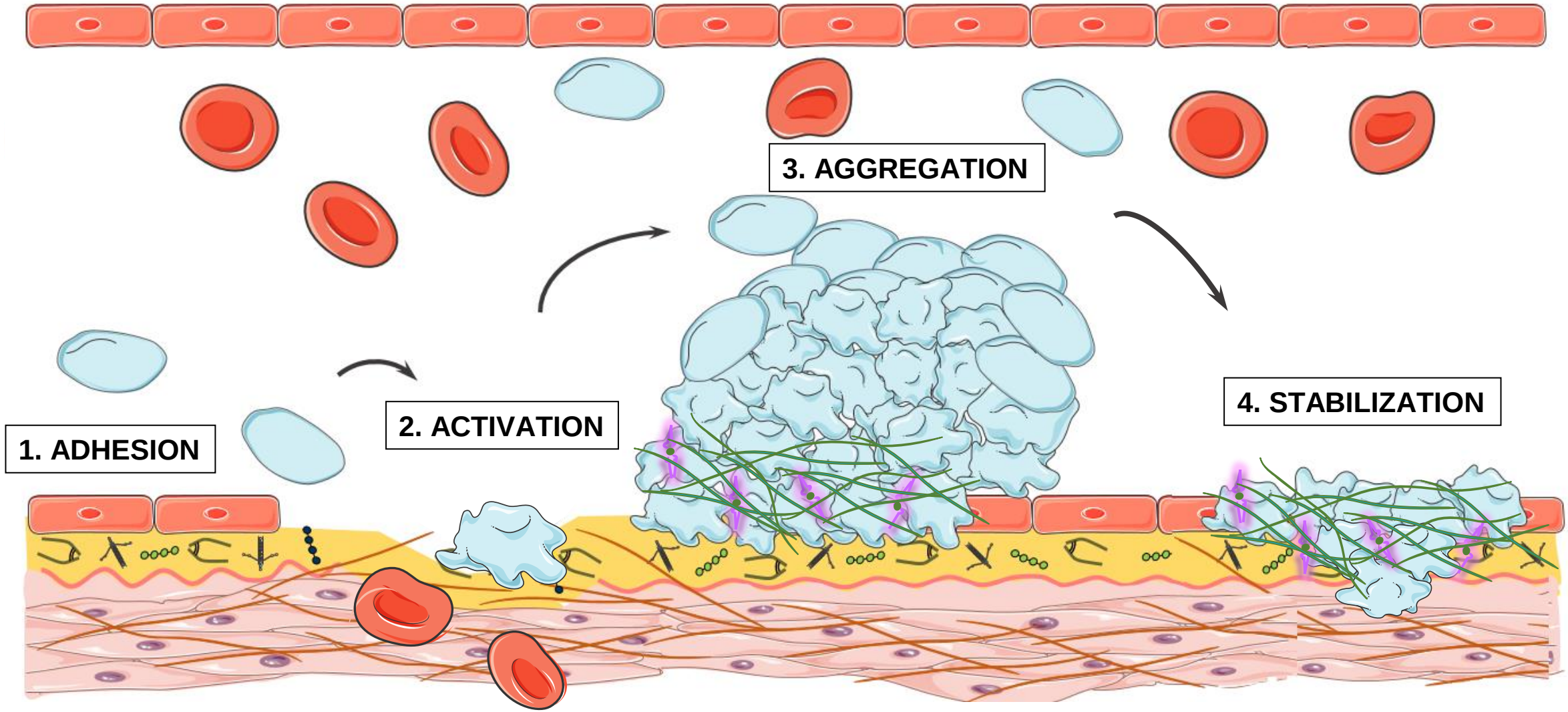
10 -12 MAI 2023

Fibrin plays a central role in avoiding an excessive hemostatic response at site of vessel injury

Yakusheva Alexandra

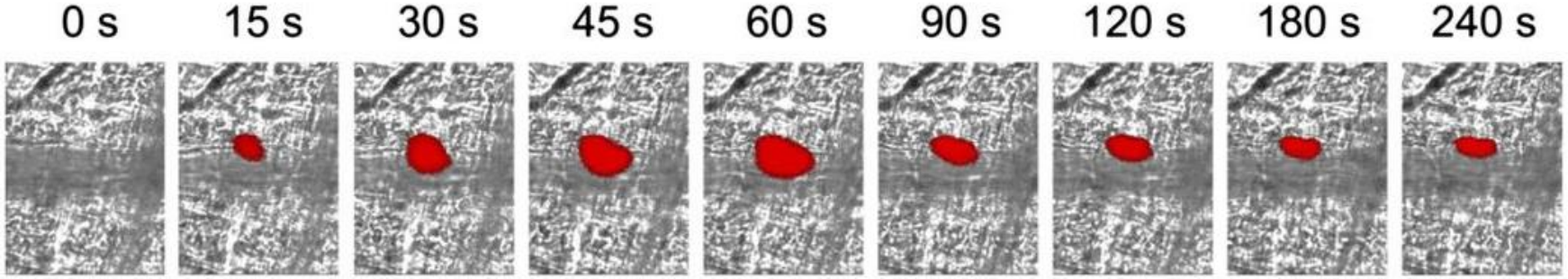
INSERM UMR_S1255
« Biologie et pharmacologie des plaquettes sanguines : hémostase, thrombose, transfusion »

Platelet response to the vessel injury



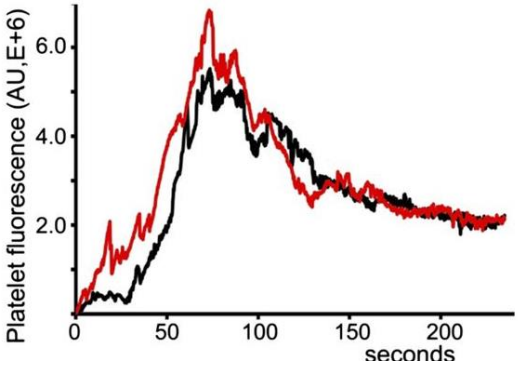
The dynamic of thrombus formation

Laser-injury in the cremaster



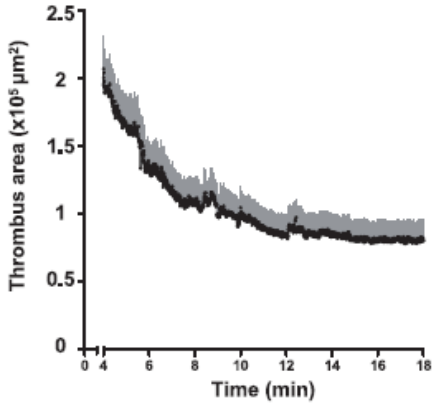
Alexey Kolyada et al. Blood 2014

Laser-injury in the cremaster



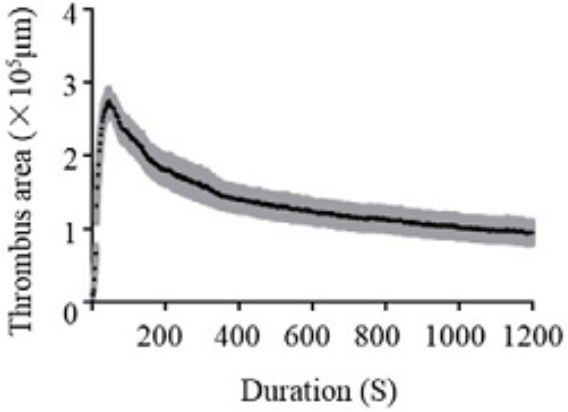
Alexey Kolyada et al. Blood 2014

Needle-injury of the carotid



Hechler et al. JTH 2011

Mechanical-injury of the aorta

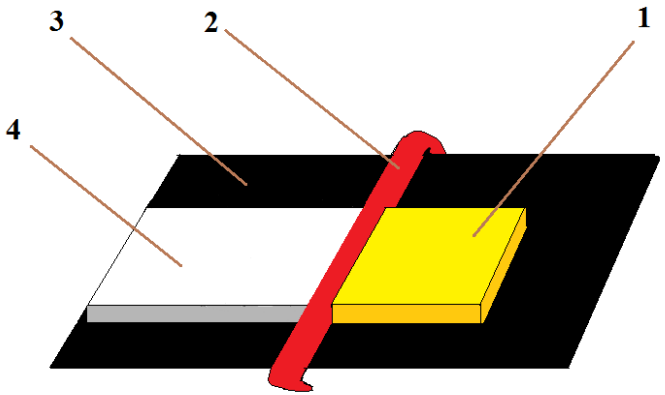


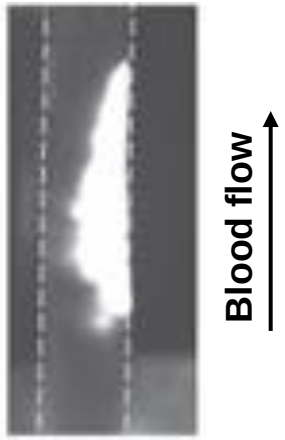
Tang et al. Thromb Res 2016

Question: How is the response to vascular injury arrested after only few minutes?

In vivo models of vascular injury

$FeCl_3$-injury of the carotid artery





Eckly et al., J Thromb Haemost (2011)

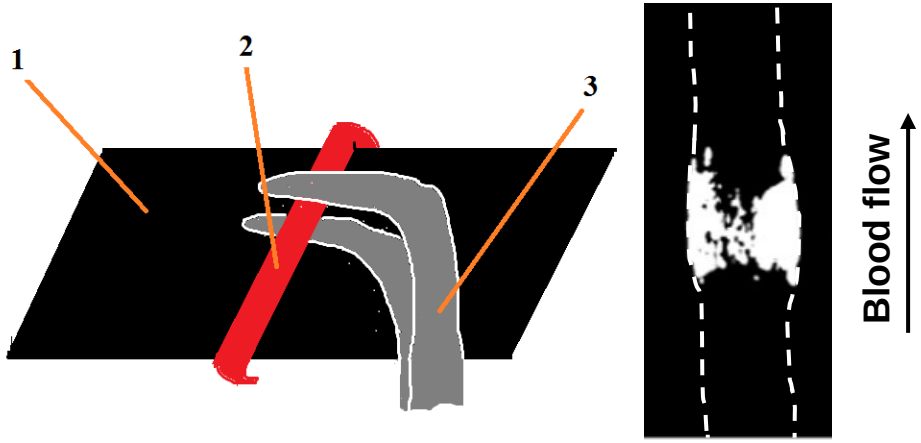
1 – a Whatmann filter paper with 7.5% FeCl3;

2 – carotid artery;

3 – black plastic rectangle;

4 – a Whatmann filter paper 5x3 with saline.

Mechanical-injury of the aorta



1 – black plastic rectangle;

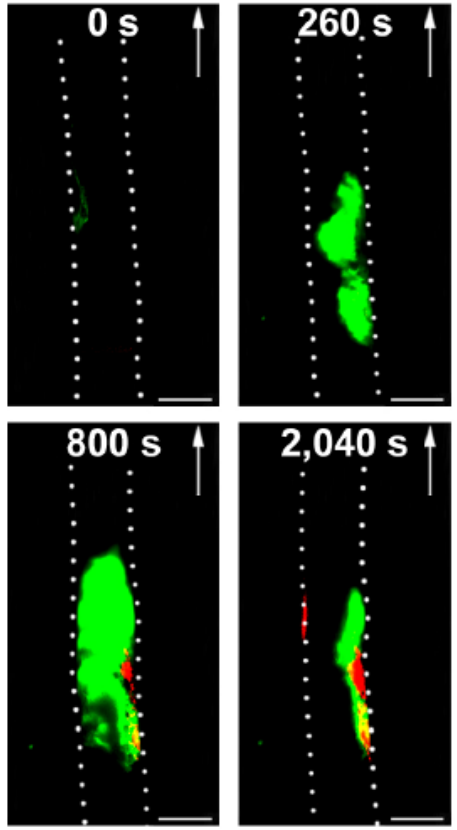
2 – aorta;

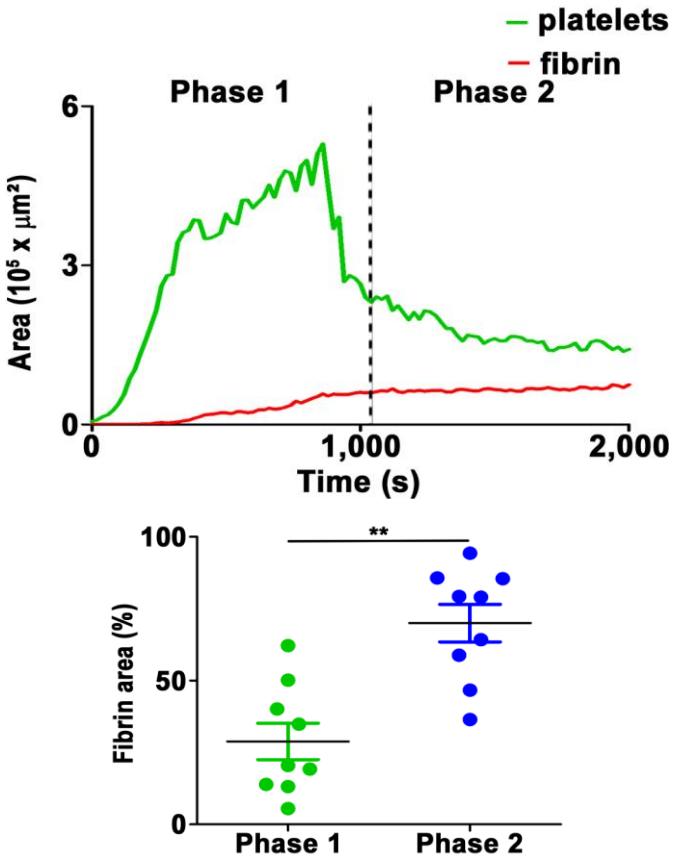
3 –forceps.

10-12 MAI 2023
CONGRÈS FRANÇAIS d'HÉMOSTASE
SAINT-MALO Palais des Congrès Le Grand Large

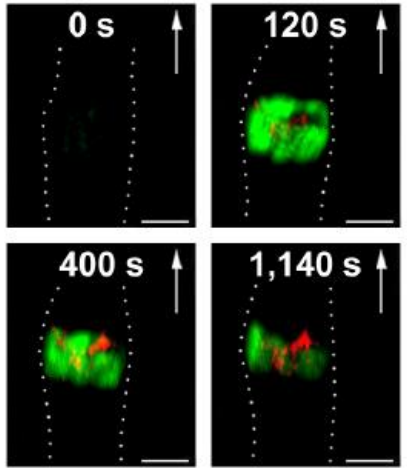
Link between fibrin formation and the arrest of the hemostatic responses

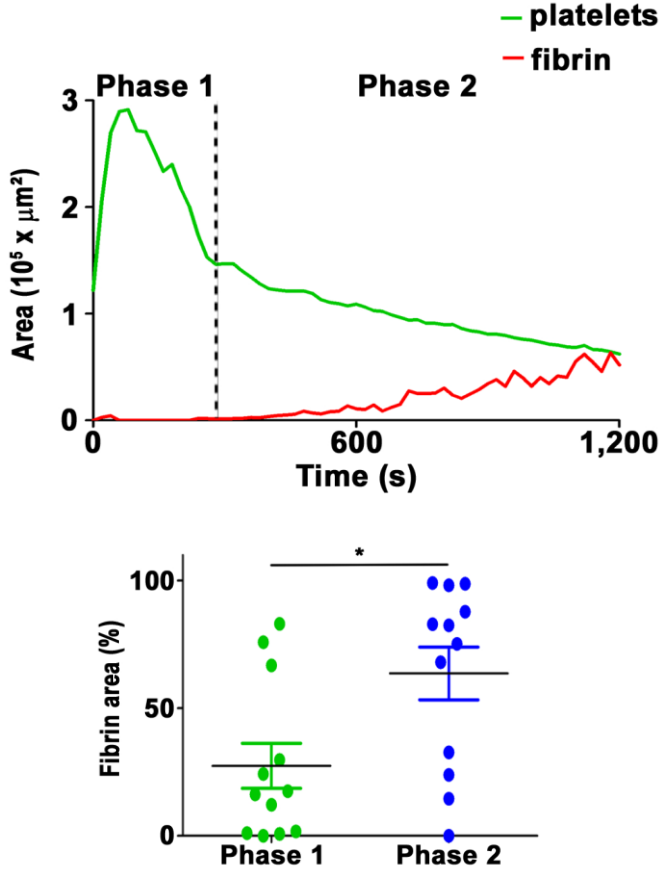
$FeCl_3$-injury model





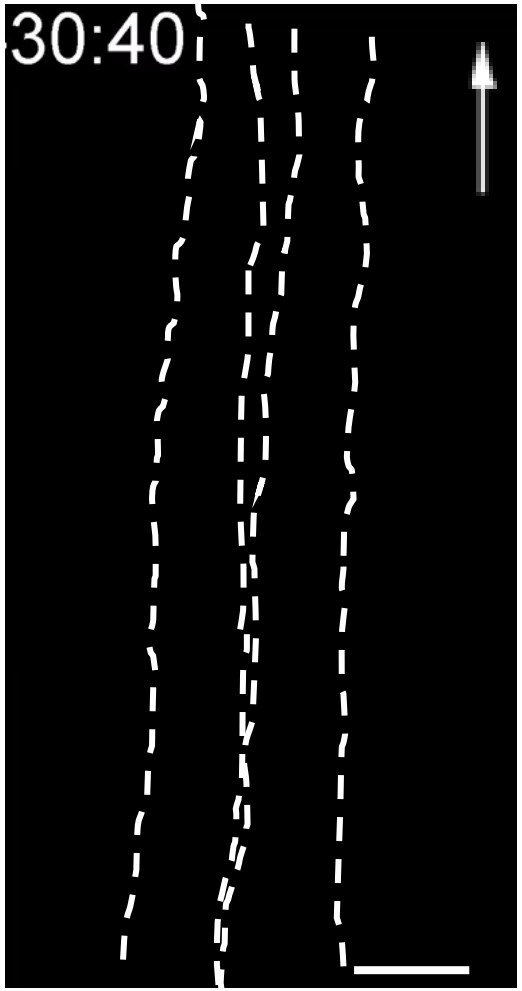
Mechanical-injury model

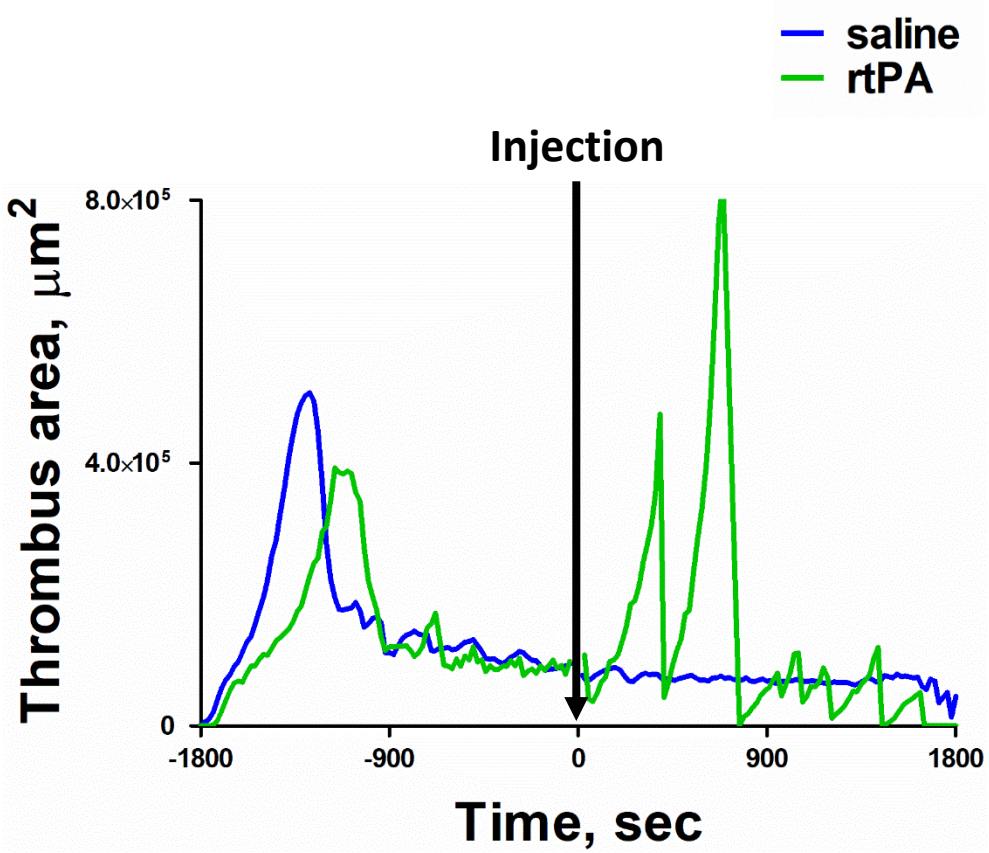


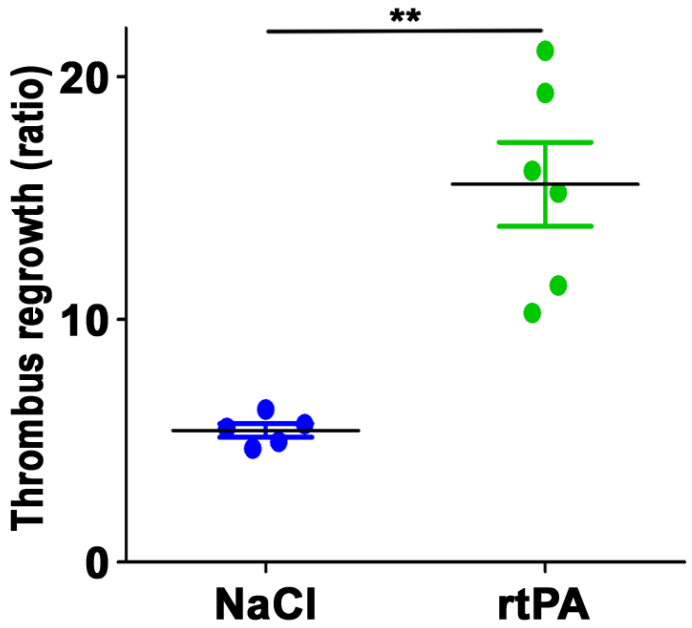


Hypothesis: Does fibrin contribute to the prevention of the repeat of the hemostatic response ?

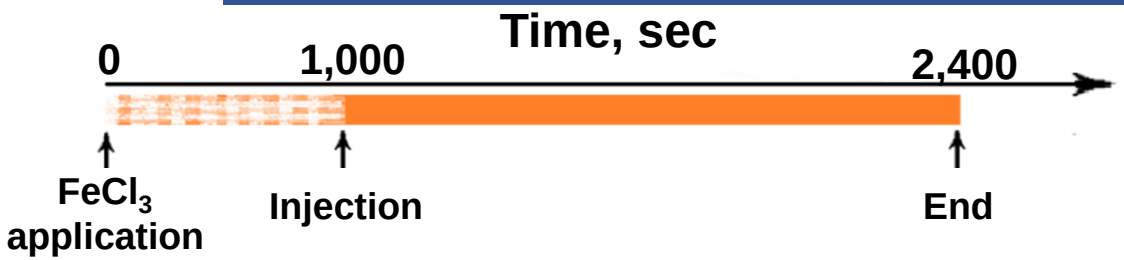
Fibrin stops the process of platelet recruitment

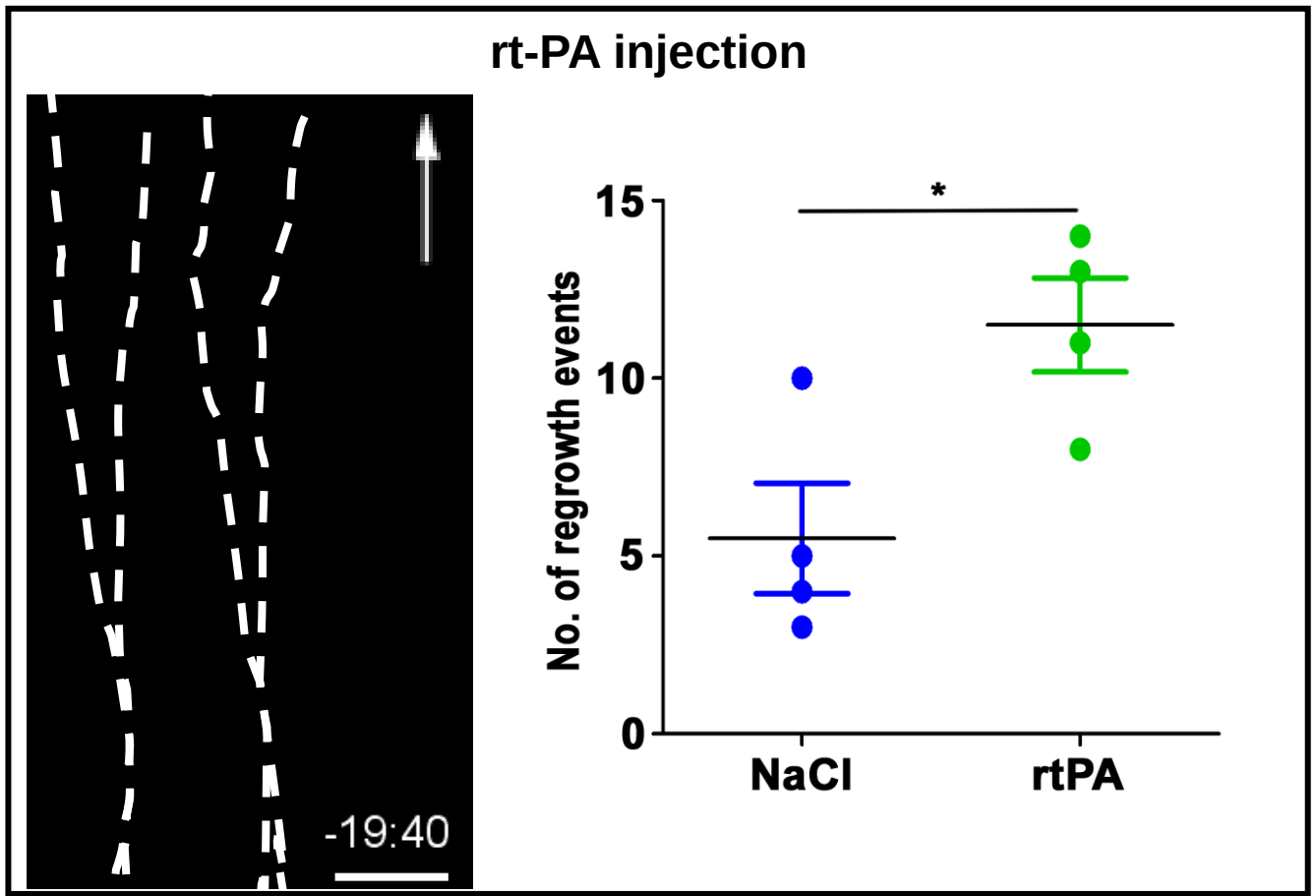


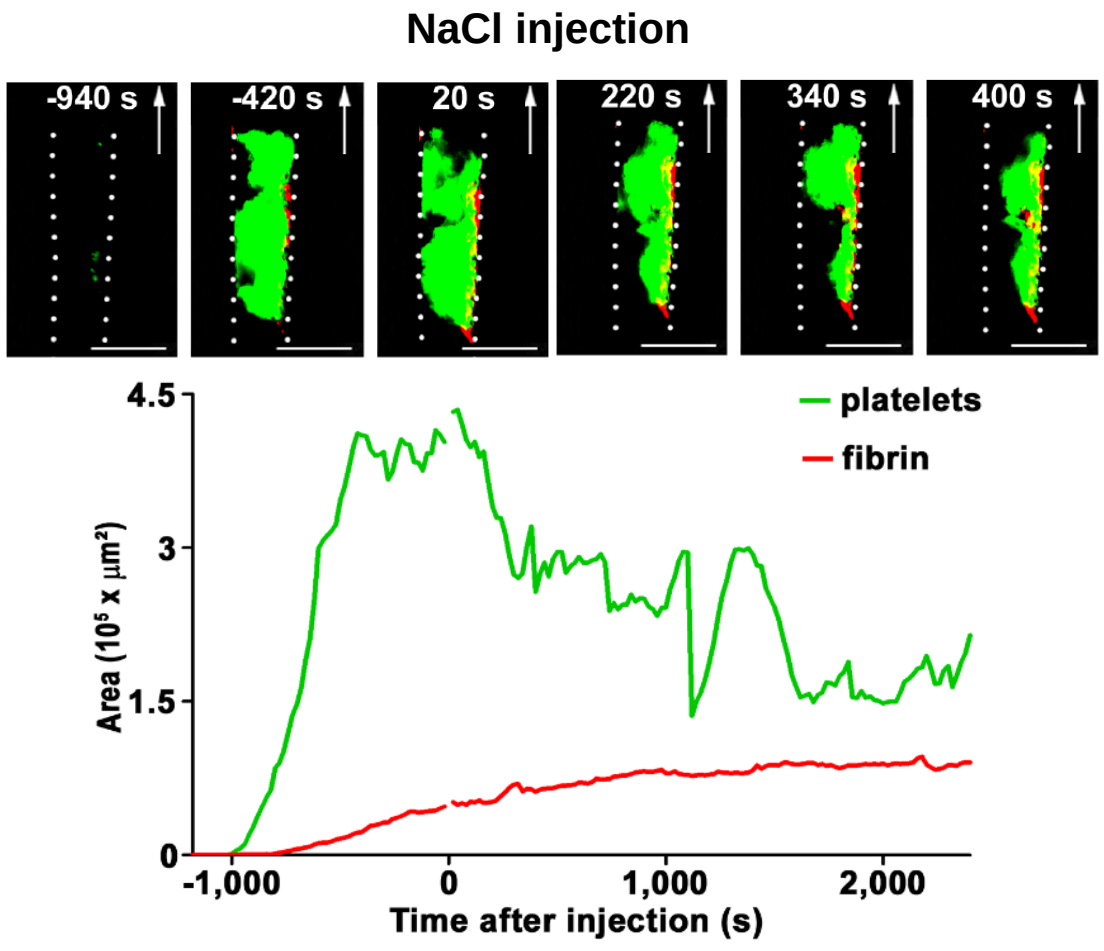




Delay in the hemostatic response when fibrin does not form: pharmacological approach

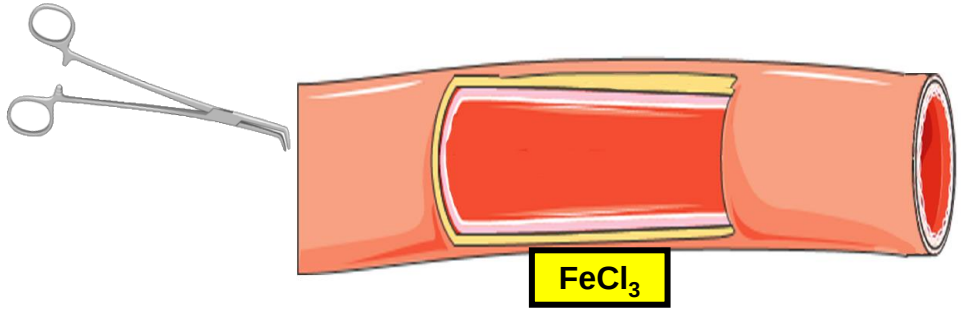


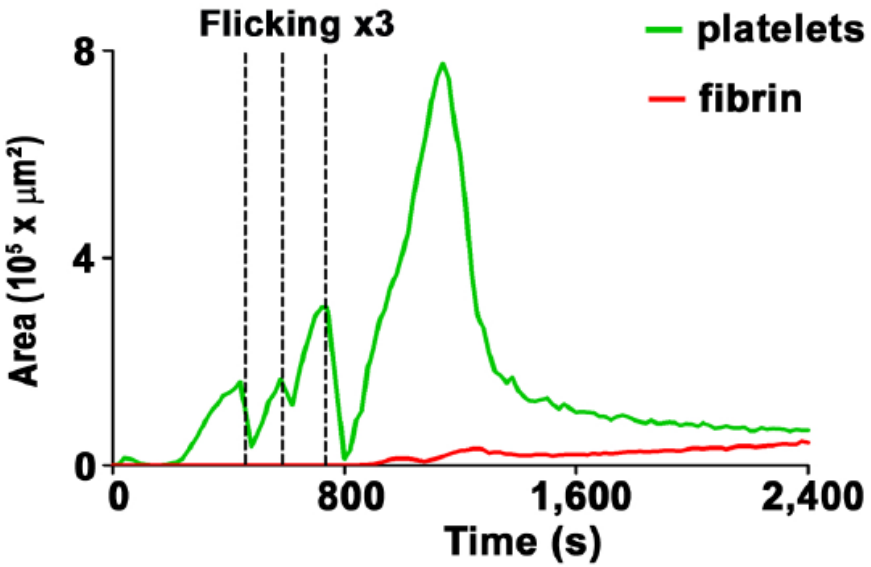


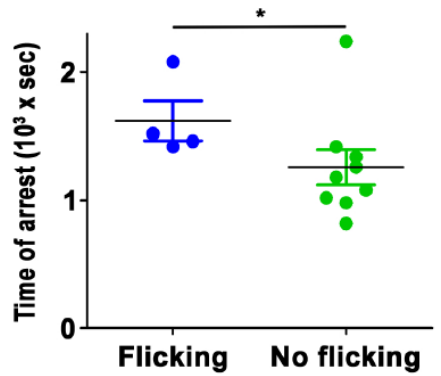


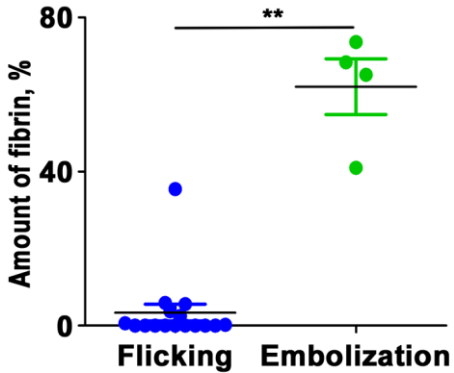
Delay in the hemostatic response when fibrin forms with a delay: mechanical approach

$FeCl_3$-injury model with of carotid artery with forceps

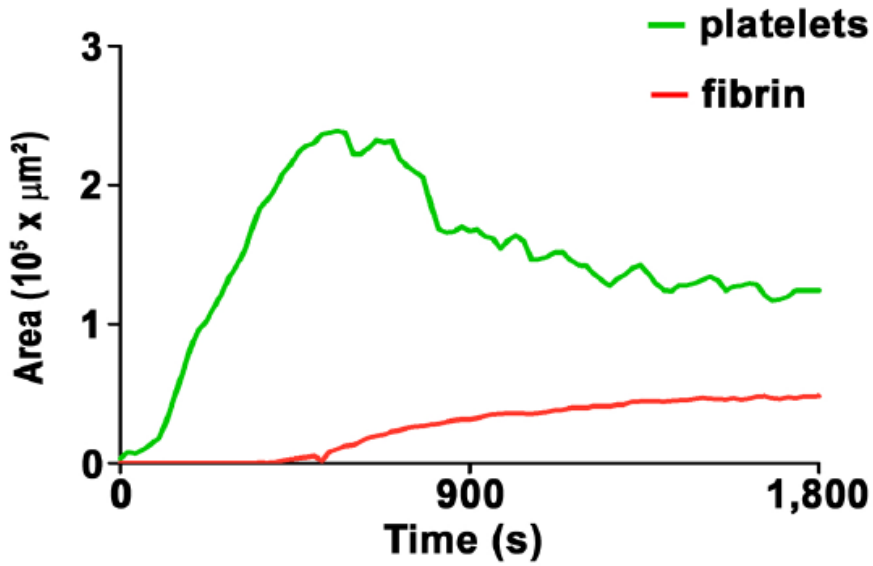








Ctrl (no flicking)

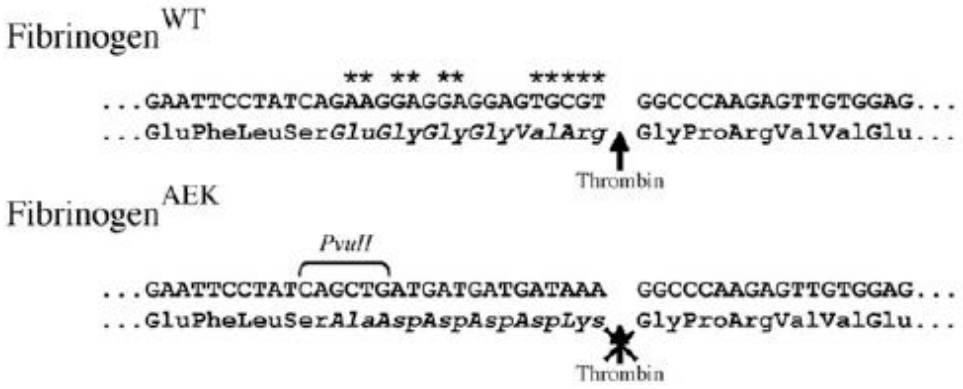


Delay in the hemostatic response when fibrin does not form: genetic approach

- **Normal behavior and physical appearance**
- **Homozygous are unable to sustain pregnancies**

FibAek mice

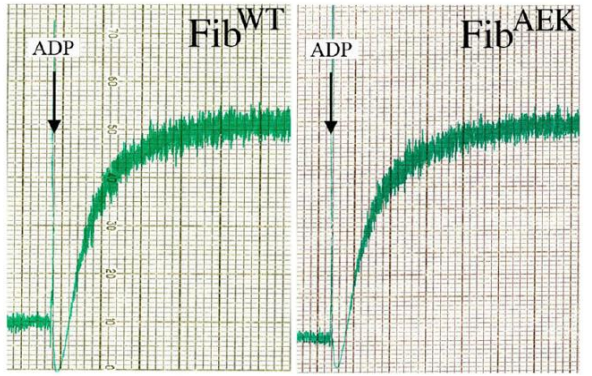
Mutation in the fibrinopeptide sequence of the Aa chain



Hematologic profile

	Fib^{WT} (N = 6)	Fib^{AEK} (N = 6)
WBC ($\times 10^9$/L)	4.95 ± 1.7	4.79 ± 1.8
RBC ($\times 10^{12}$/L)	8.92 ± 0.5	9.02 ± 0.5
Hemoglobin (g/dL)	12.15 ± 0.7	12.23 ± 0.5
Hematocrit (%)	51.52 ± 3.7	50.80 ± 2.9
Platelets ($\times 10^9$/L)	1005 ± 157	892 ± 145
PT (seconds)	12.5 ± 0.2	>180
aPTT (seconds)	29.3 ± 2.7	>300
Thrombin time (seconds)	14.9 ± 1.1	>90

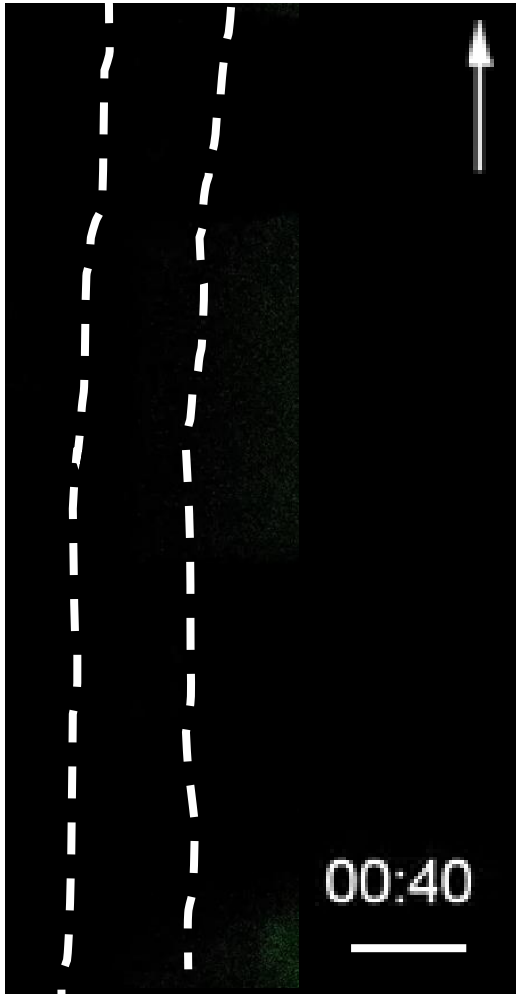
Platelet aggregation

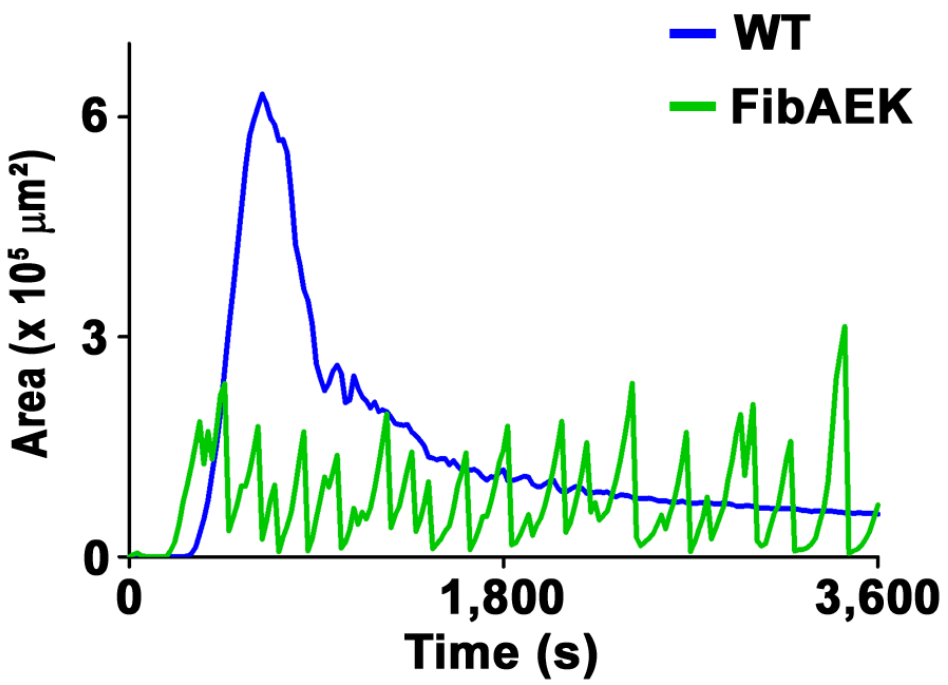


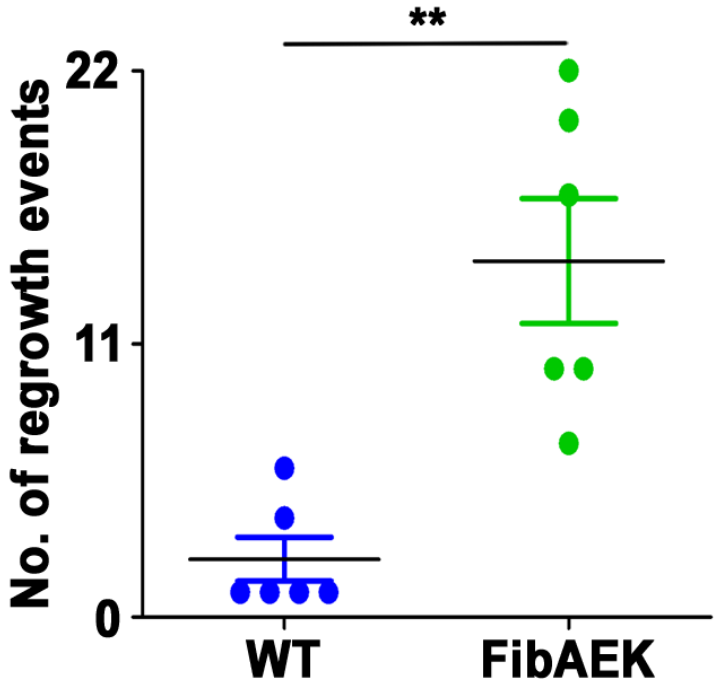
Flick et al. Blood 2015

Delay in the hemostatic response when fibrin does not form: genetic approach

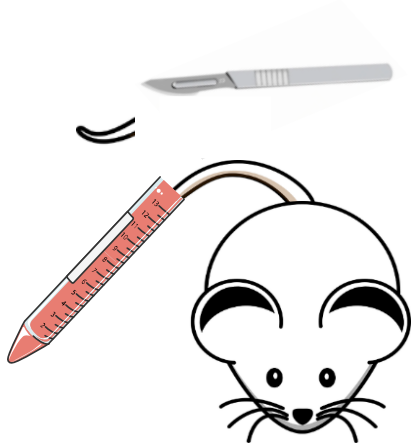
$FeCl_3$-injury model of carotid artery

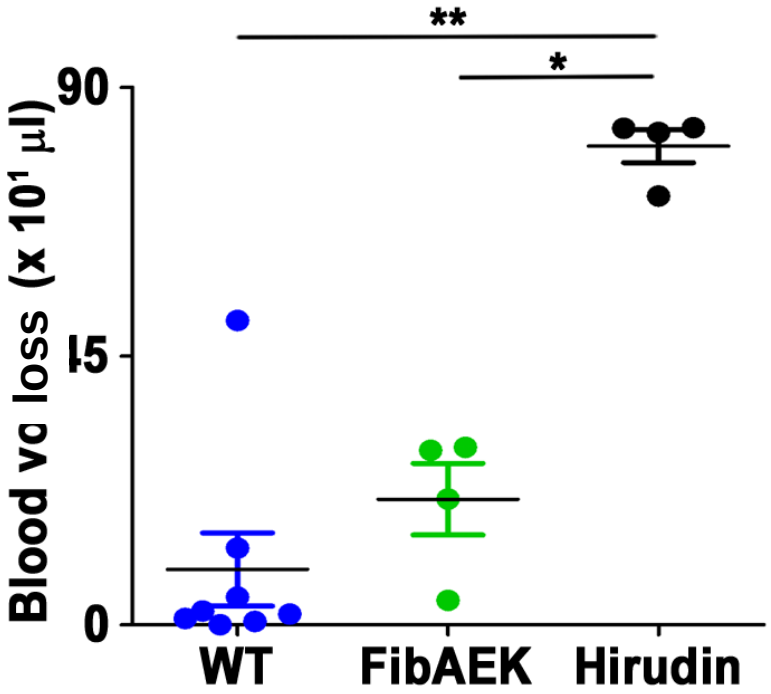


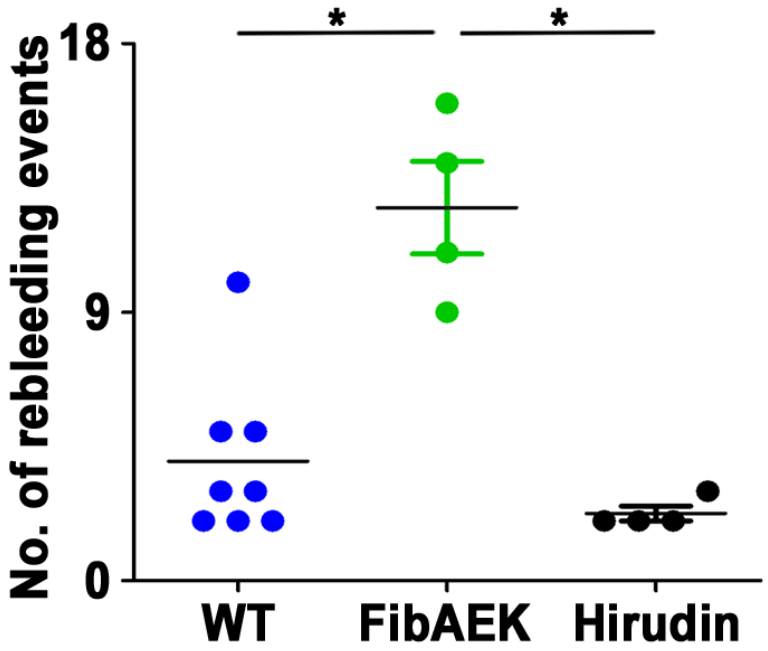




Delay in the hemostatic response when fibrin does not form: genetic approach

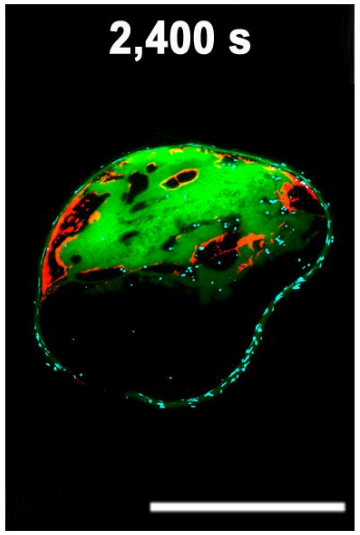




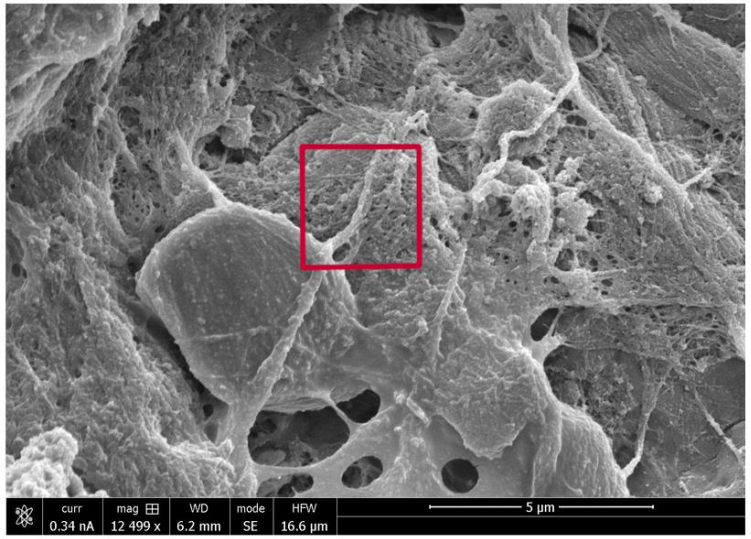


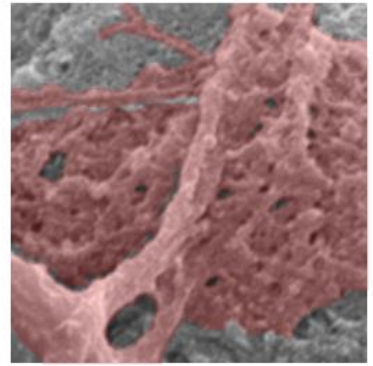
Mechanism: fibrin layer is poorly adhesive

$FeCl_3$-injury model of carotid artery



platelets
fibrin
DNA



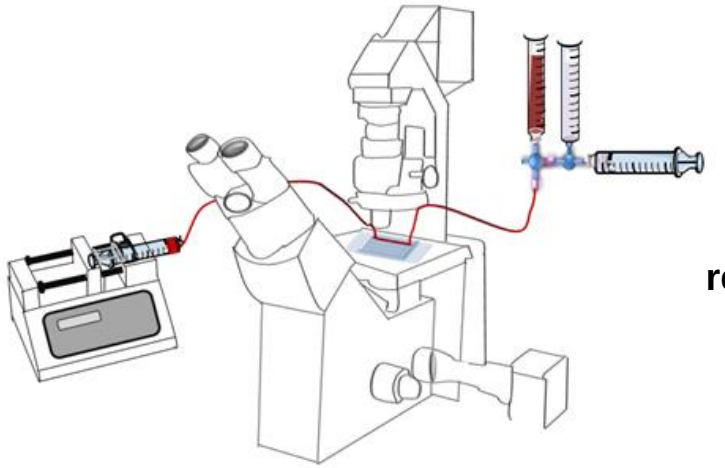


fibrin

The residual platelet plug is rich in fibrin, which reaches its top to form a layer

A fibrin layer prevents thrombus growth *in vitro*

***In vitro* flow system**

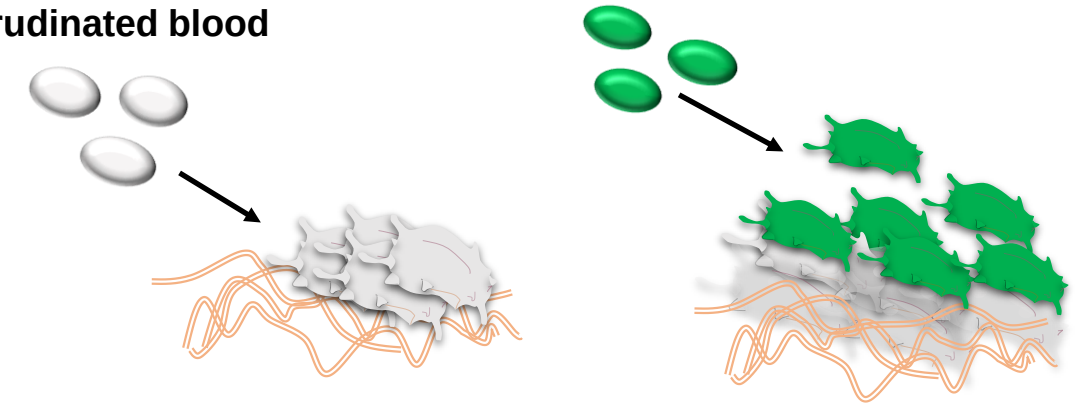


Usman Ahmed

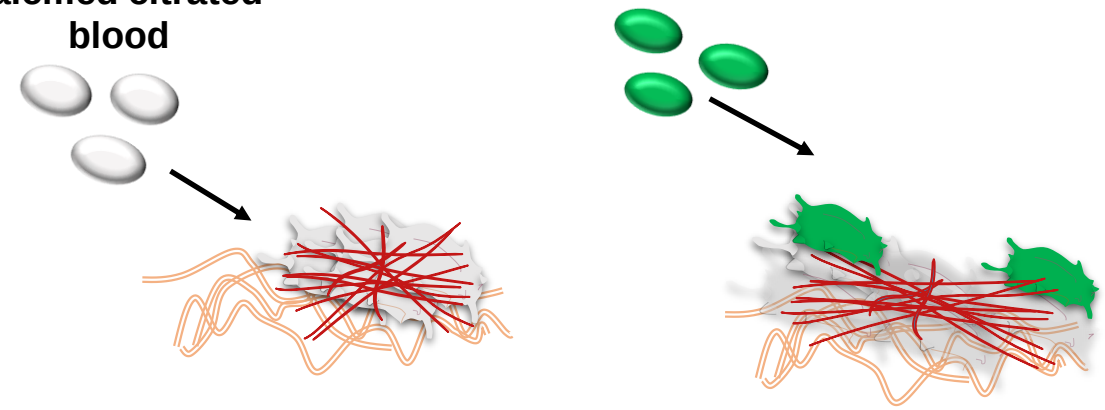
1st Population:

hirudinated blood

2st Population:



recalcified citrated blood



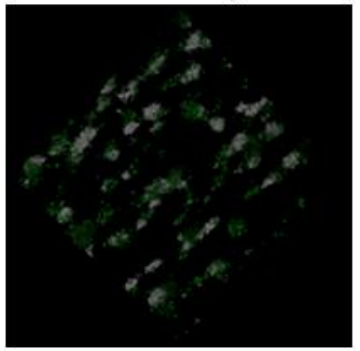
fibrin

platelets: 2nd population

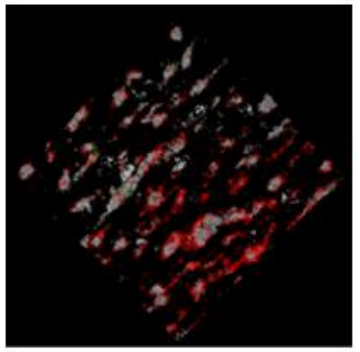
platelets: 1st population

Overlay

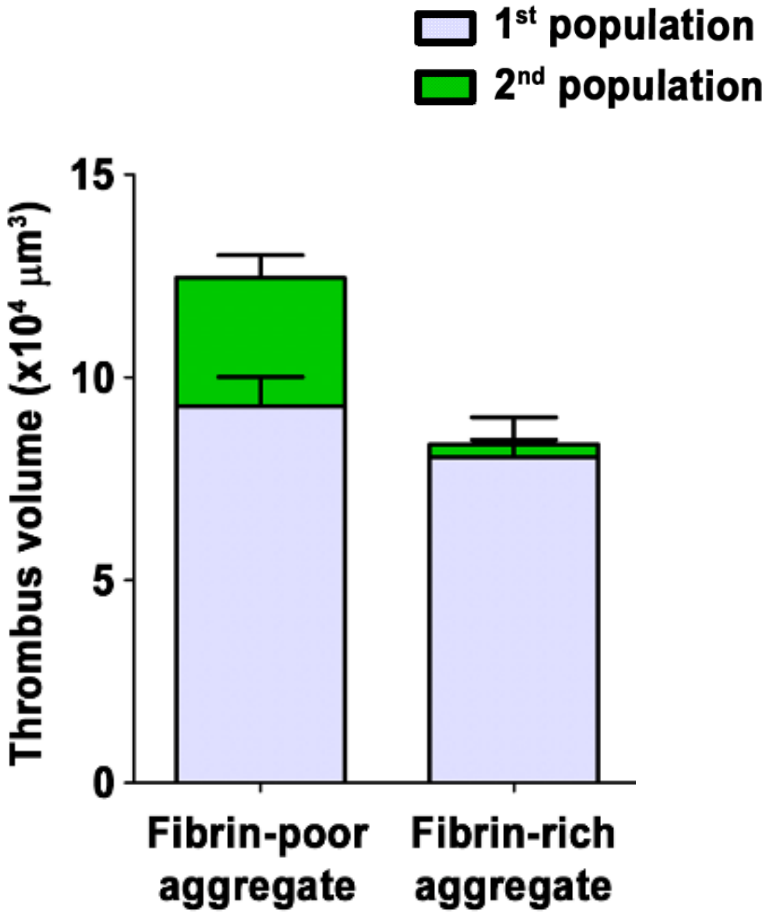
Fibrin-poor aggregate

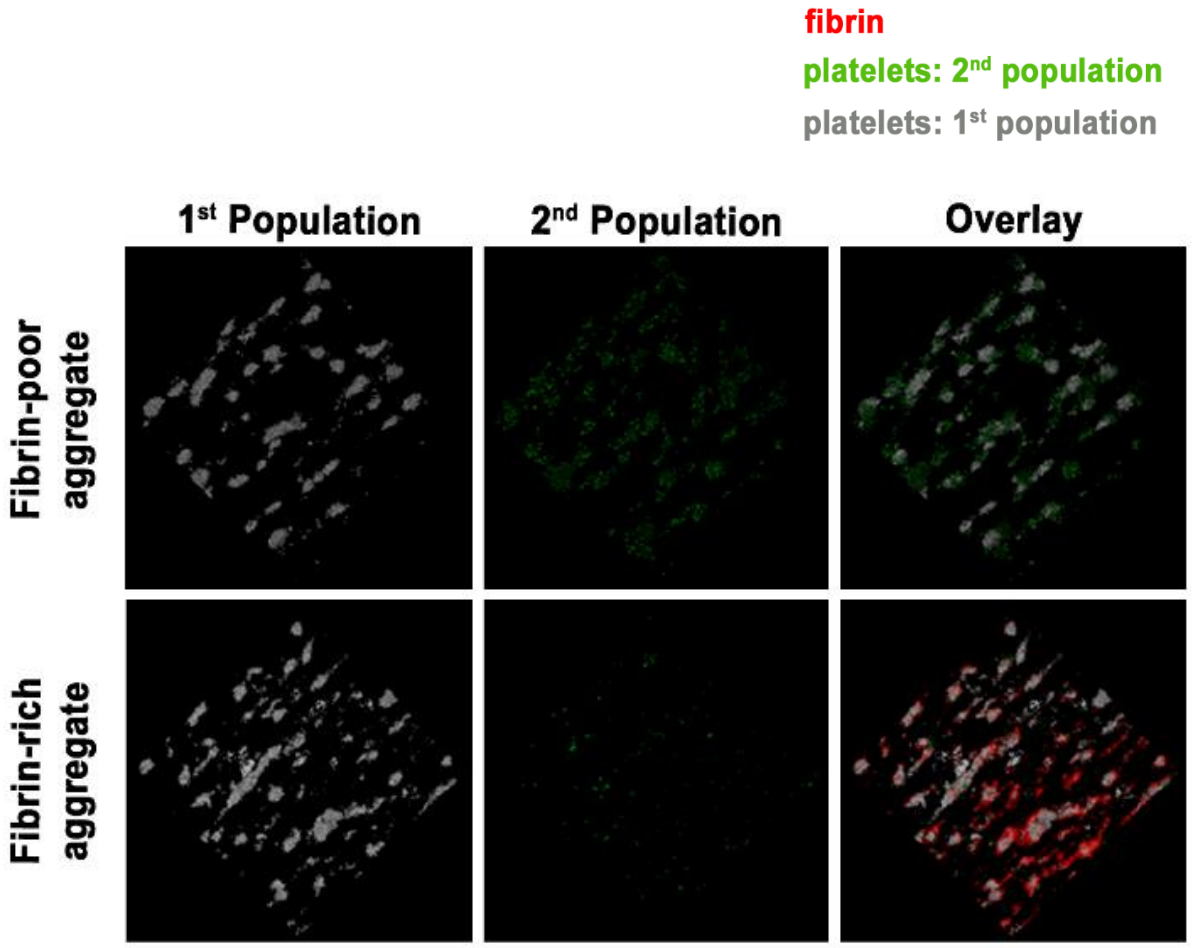


Fibrin-rich aggregate



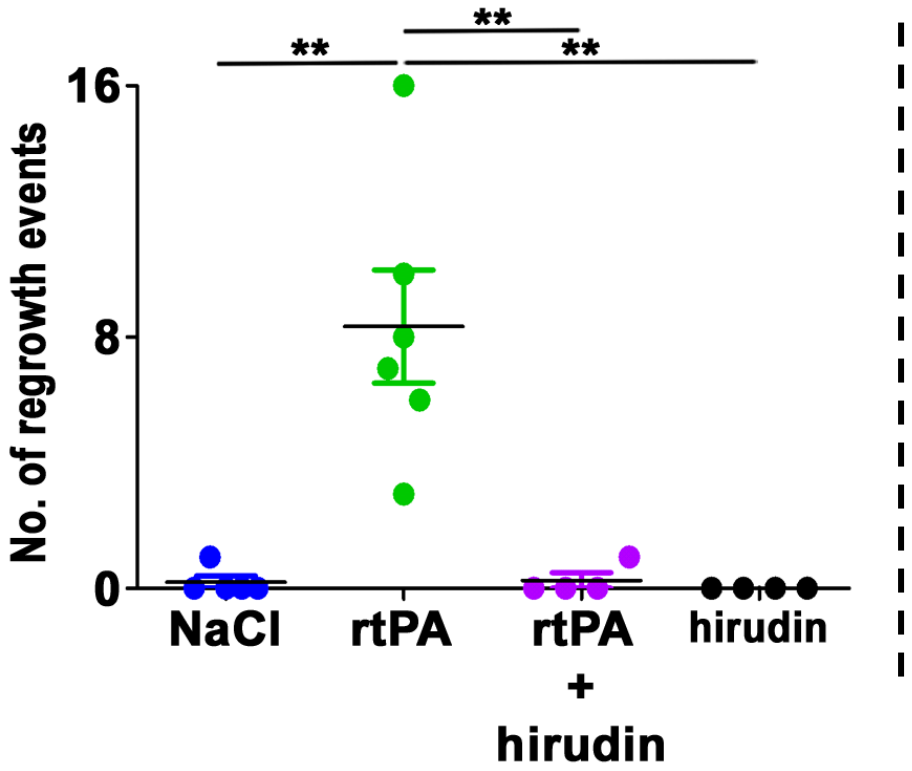
A fibrin layer prevents thrombus growth *in vitro*



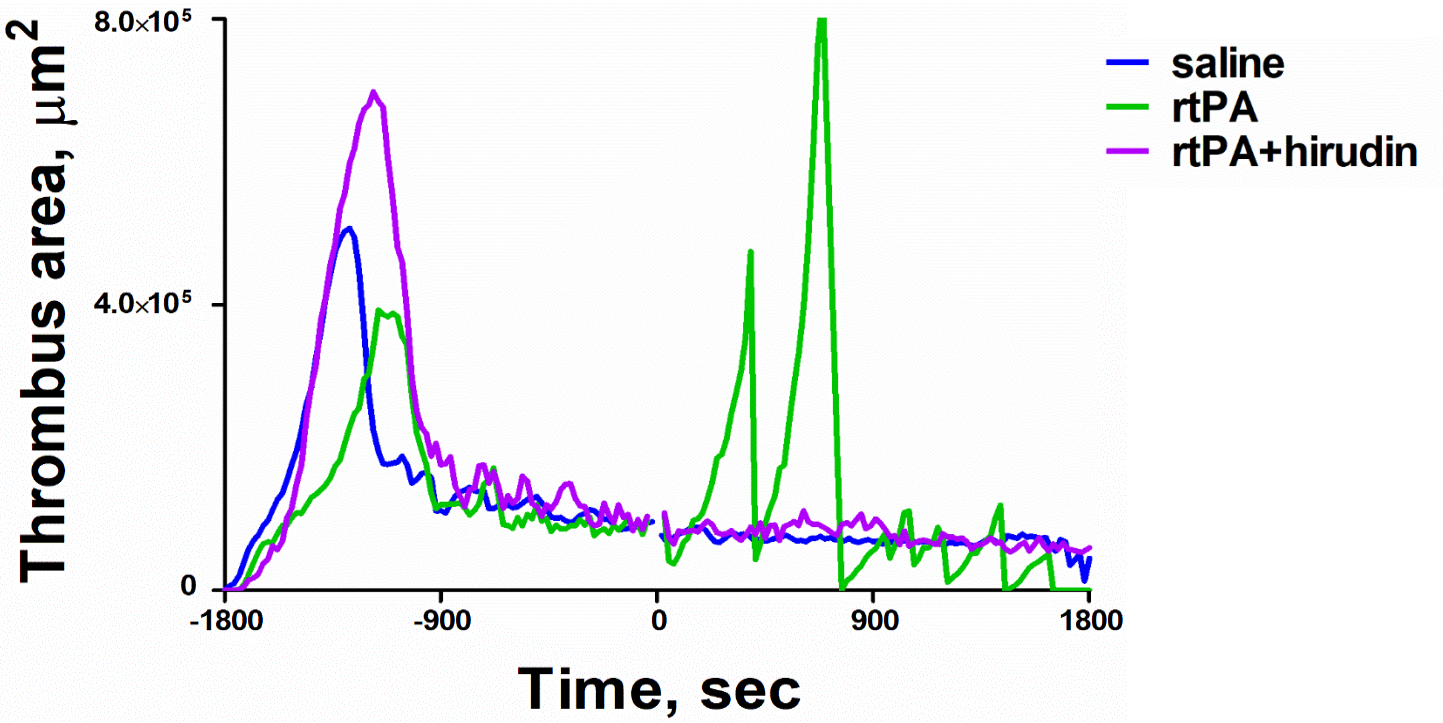


A fibrin layer exhibits a protective function and stops platelet adhesion and aggregaion

Mechanism: fibrin traps thrombin limiting its diffusion



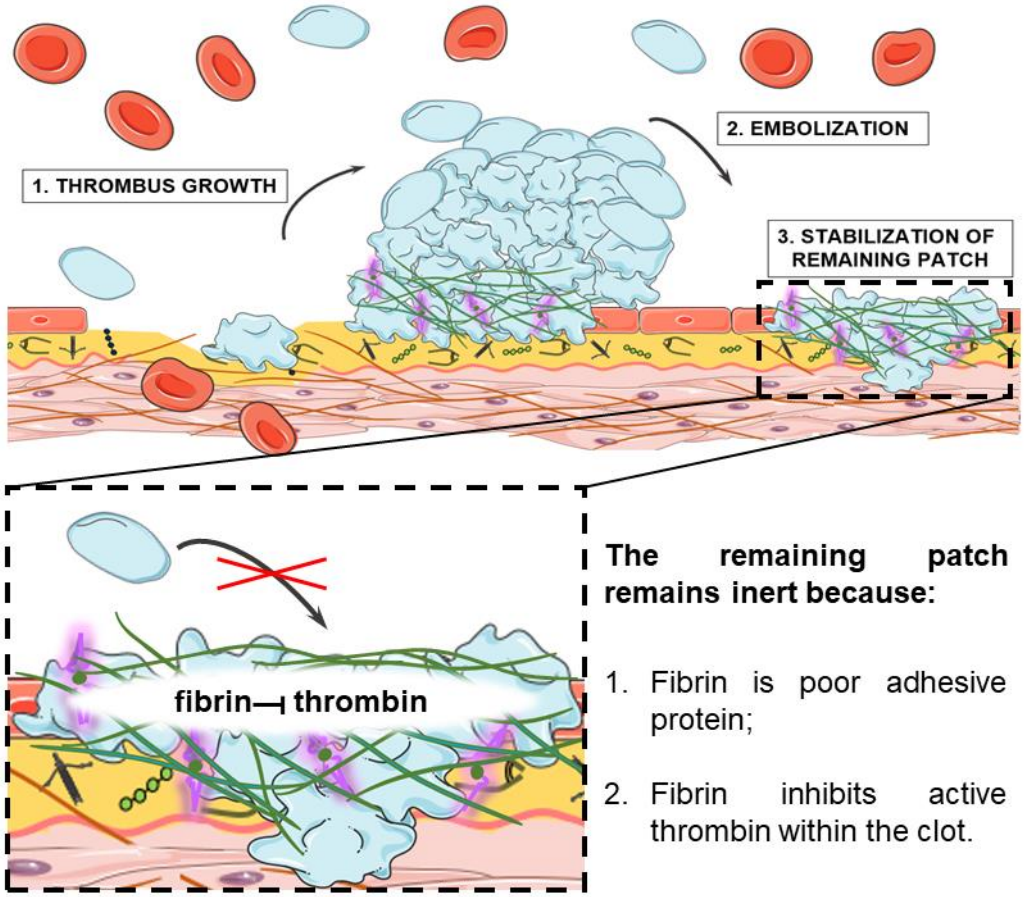
$FeCl_3$-injury model with injection



While rtPA started to lyse fibrin a regrowth was observed which was due to thrombin as this effect disappeared in the presence of hirudin

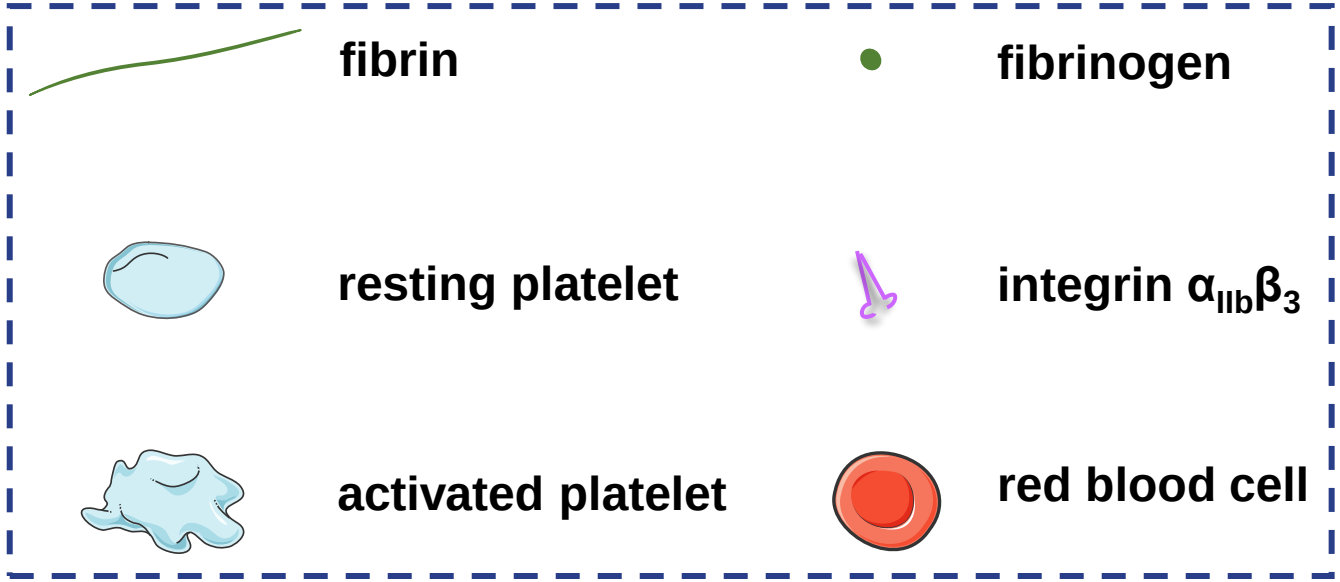
Conclusion

Fibrin is a major player in avoiding excessive platelet plug formation at site of vascular damage



The remaining patch remains inert because:

1. Fibrin is poor adhesive protein;
2. Fibrin inhibits active thrombin within the clot.



Acknowledgement

UMR_S1255 EFS Grand-Est, Strasbourg

Pierre H. Mangin

Muhammad Usman Ahmed

Catherine Bourdon

Kim Jouffroy

Charlotte Fauth

Clarisse Mouriaux

Center for Theoretical Problems of Physicochemical Pharmacology, Moscow

Mikhail A. Panteleev

Cincinnati Children's Hospital Medical Center, Cincinnati

Matthew J. Flick

Thank you for your attention!

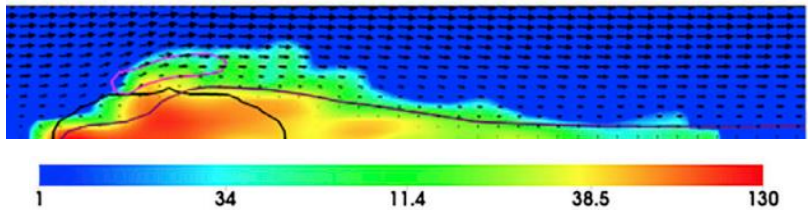
Conclusion

1. **Fibrin is a major player in avoiding excessive platelet plug formation at site of vascular damage**
2. **Potential mechanism:**
   - **Fibrin limits platelet accumulation on a fibrin-rich plug**
   - **Fibrin limits the pro-hemostatic response of thrombin generated in a plug**

Thank you for your attention!

What is the mechanism by which fibrin arrests of thrombus regrowth?

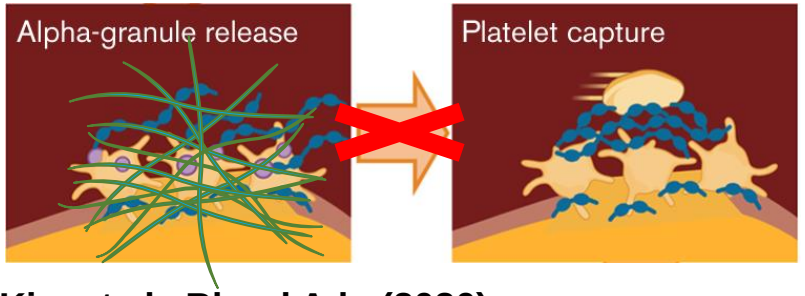
Fibrin decreases the permeability-porosity of thrombus



Velocity (cm/s)

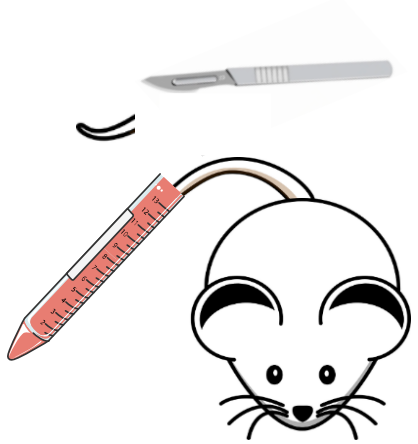
Du et al., Biophys J (2020)

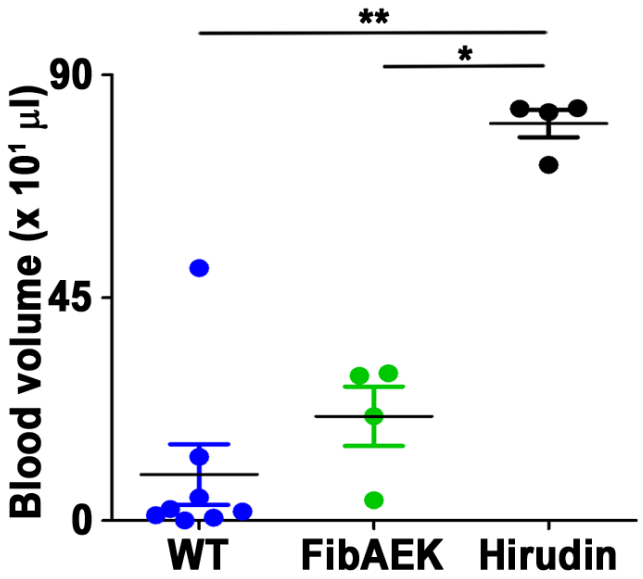
Fibrin restricts the distribution of granule

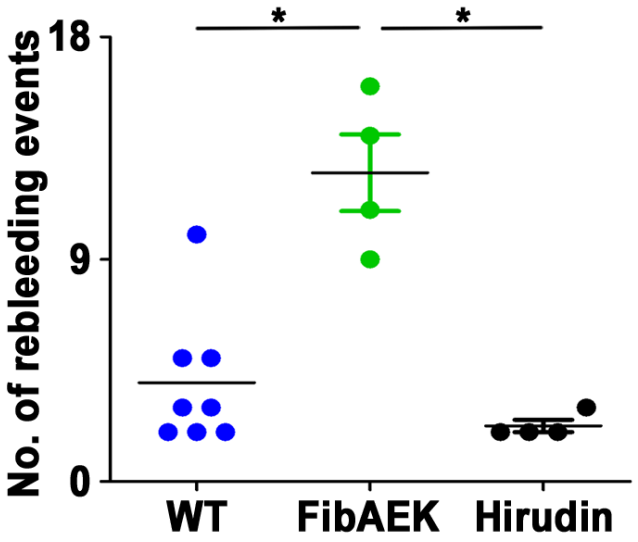


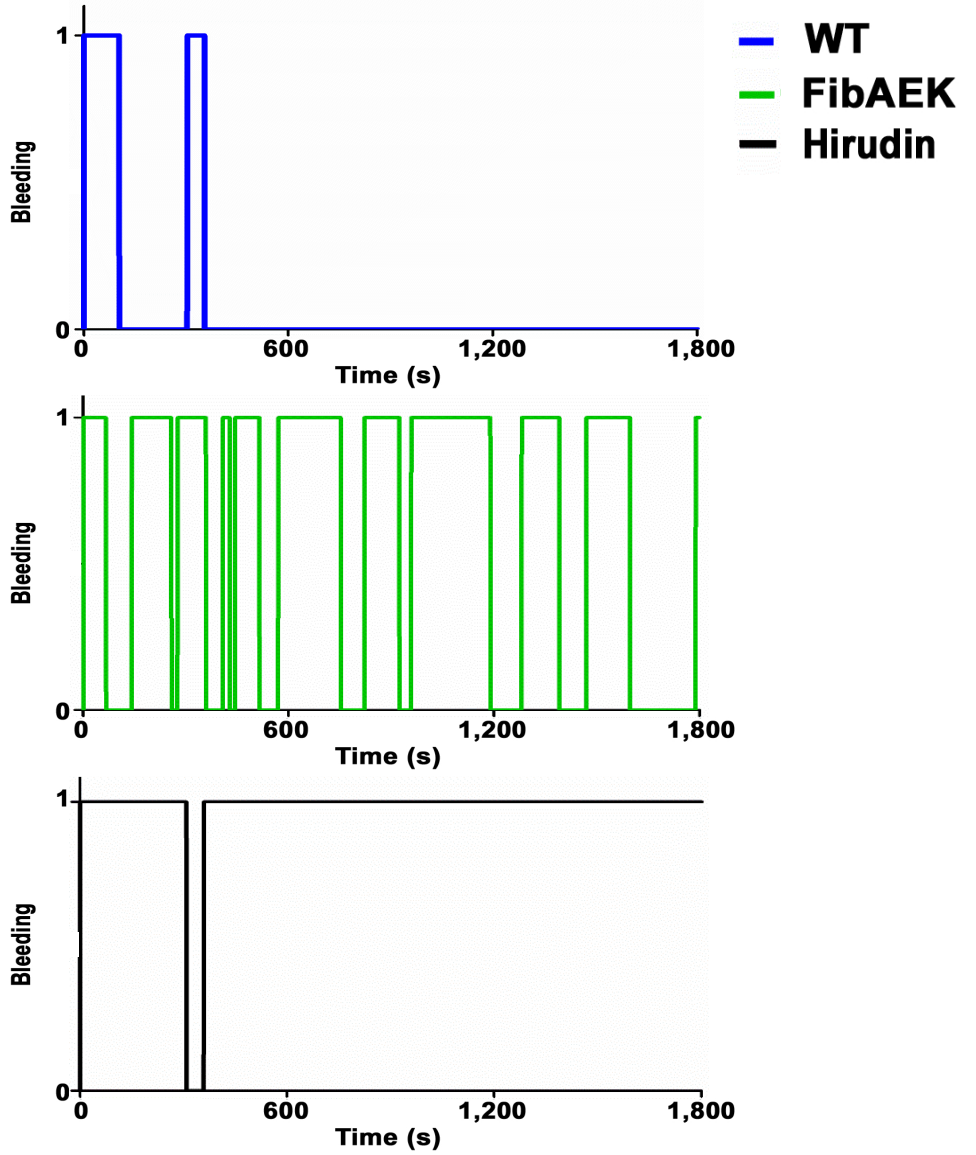
Kim et al., Blood Adv (2020)

Delay in the hemostatic response when fibrin does not form: genetic approach

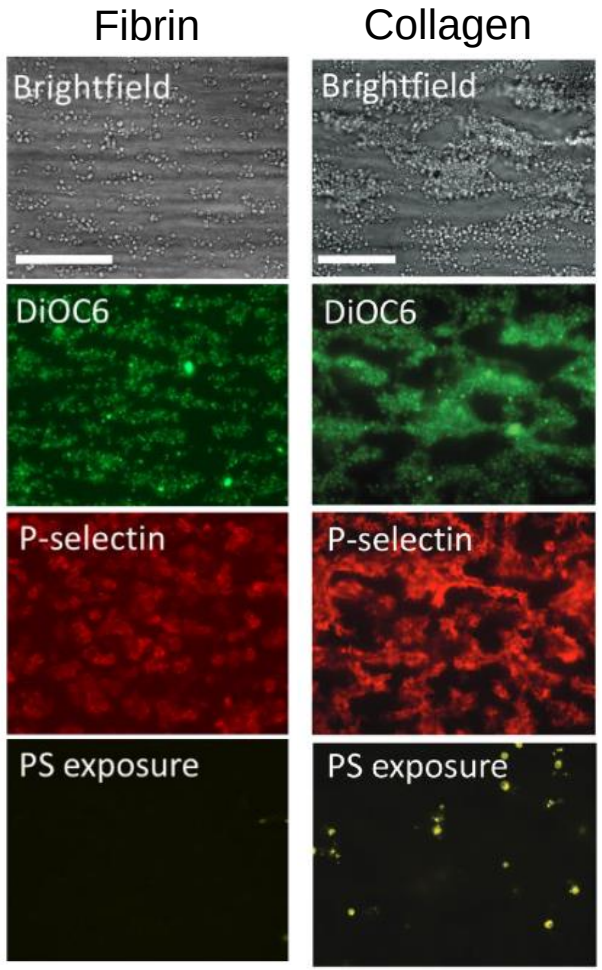






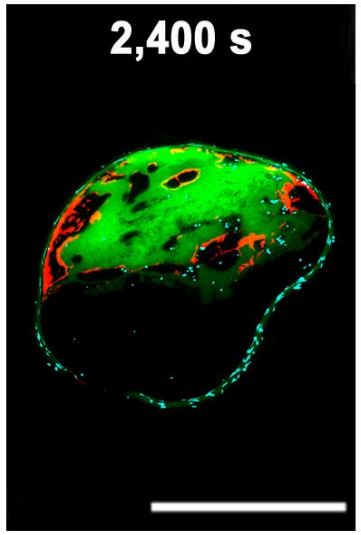


Mechanism: fibrin layer is poorly adhesive

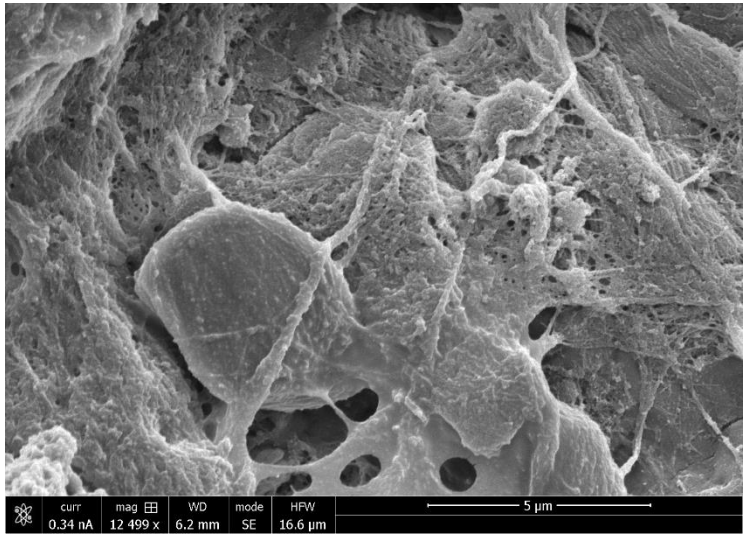


SR: 1,000 s^{-1}
Time: 10 min

$FeCl_3$-injury model of carotid artery



platelets
fibrin
DNA

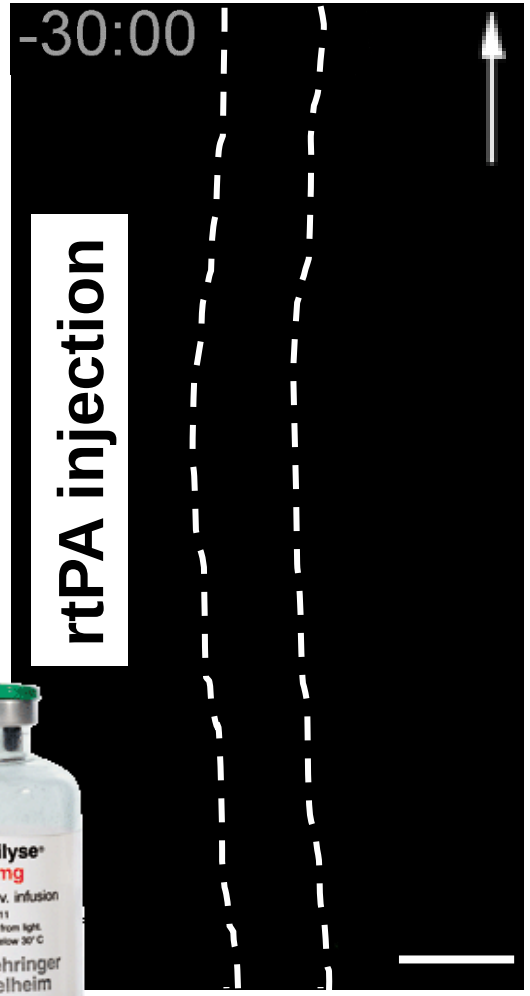


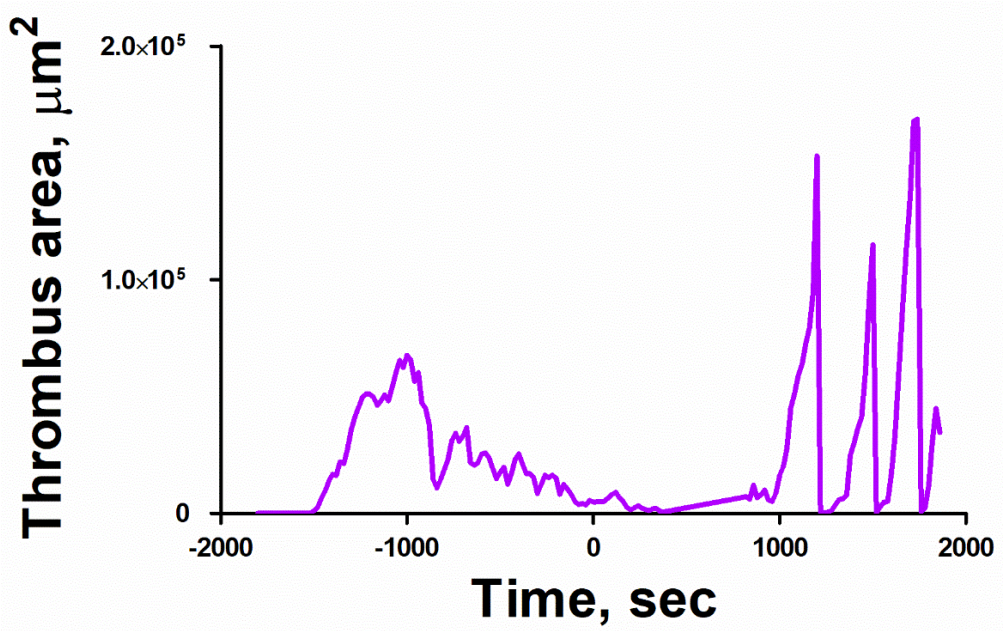
The residual platelet plug is rich in fibrin, which reaches its top to form a layer

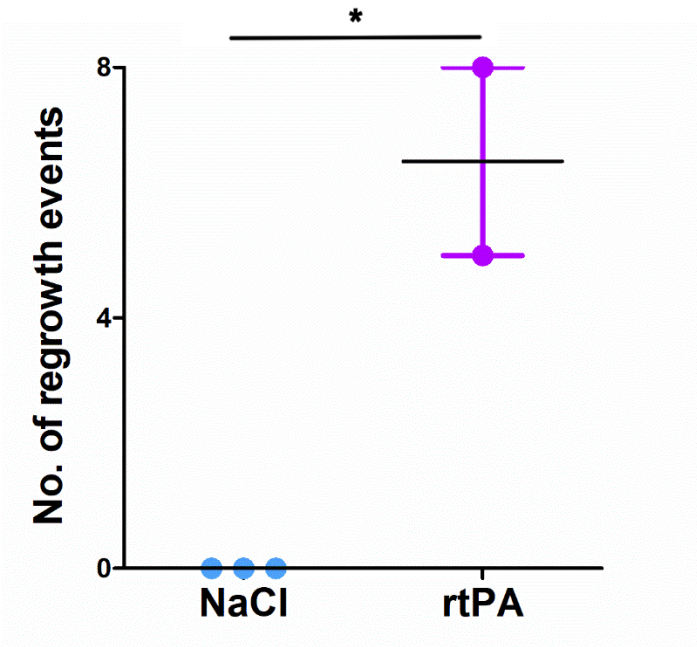
Perrella et al., ATVB (2021)

Fibrin stops the process of platelet recruitment

rtPA injection
10% bolus+ 90% injection







The efficacy and safety of tenecteplase versus alteplase

VS

	Alteplase	Tenecteplase
Fibrin selectivity	medium	high
Half-life	5 min	17 min
Dosing	bolus plus infusion	single bolus
		enhanced PAI-1 resistance

Potla et al., Int J of Emerg Med (2022)

Adverse effects of Acteplase:

- neurotoxicity;
- blood brain barrier disruption;
- intra-cerebral hemorrhage.

Kenna et al., Neurochem Res (2020)

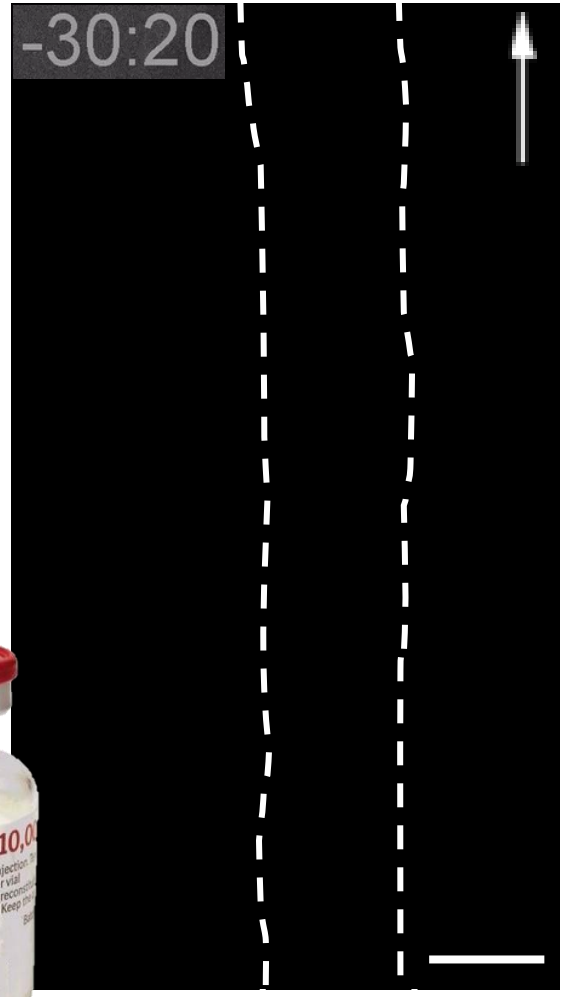
Efficiency of Tenecteplase:

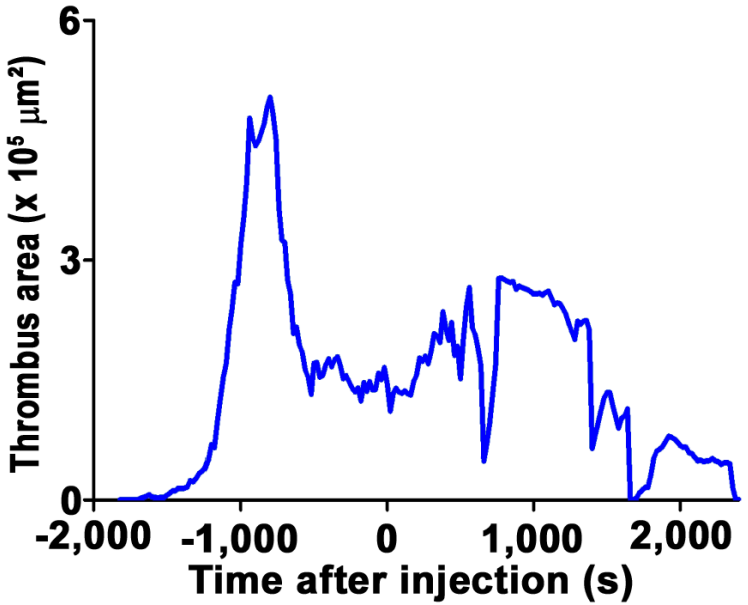
- before endovascular treatment: superior clinical efficacy;
- blood brain barrier disruption;
- acute myocardial infarction: similar rates of intracerebral hemorrhage.

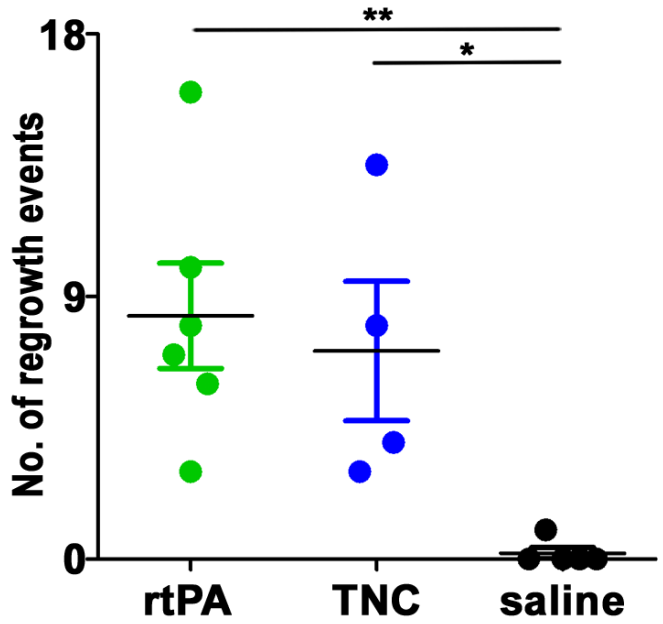
Abuelazm et al., J Thromb Thrombolysis (2023)
Teivane et al., Medicina (Kaunas) (2022)
Keragala et al., Front Neurol (2020)

Fibrin stops the process of platelet recruitment

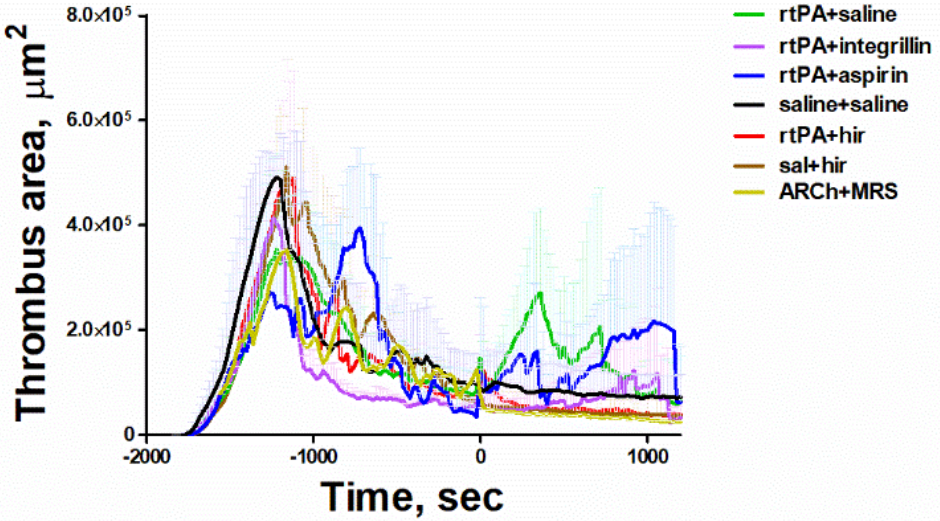
Tenecteplase (TNK) injection

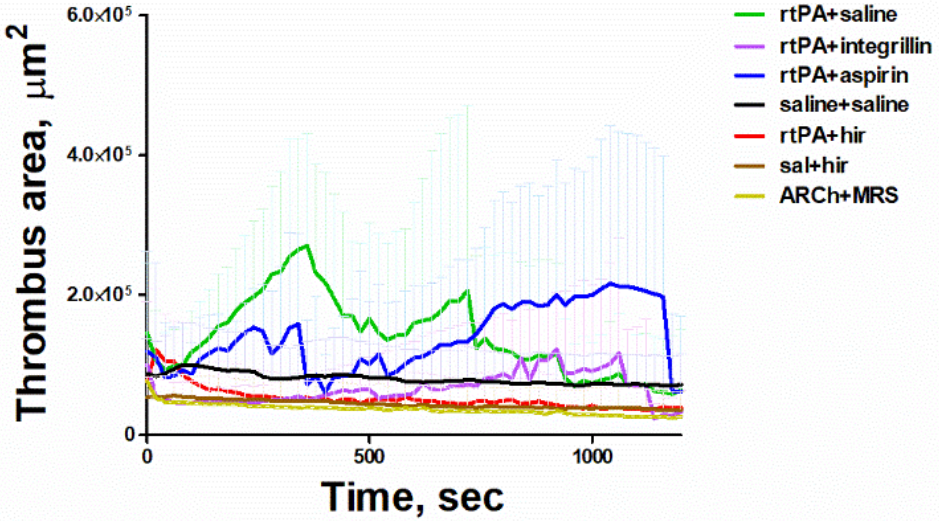


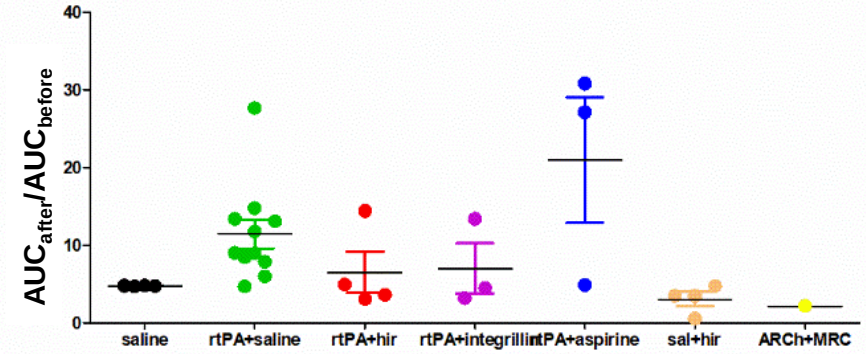


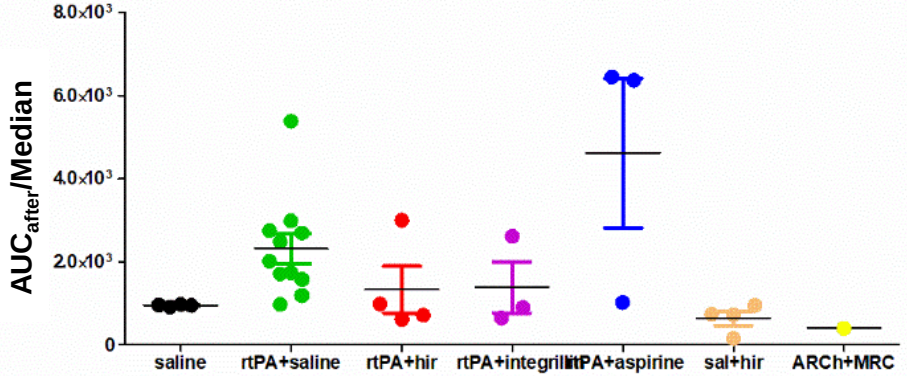


Characterization of regrowth process









rtPA 4 mg/ml
Hirudin 20mg/kg (2.5 µl/g ; 8 mg/ml)
ASA 10 mg/kg (2.5 µl/g ; 4 mg/ml)
ARCh 1 mg/kg (2.5 µl/g ; 0.4 mg/ml)
ARCl 0,06 mg/kg (2.5 µl/g ; 0.0024 mg/ml)
MRS2500 1 mg/kg (2.5 µl/g ; 0.4 mg/ml)