



11-13  
SEPT.  
2024

LILLE  
GRAND PALAIS

# CONGRÈS FRANÇAIS d'HÉMOSTASE



SFTH  
SOCIÉTÉ FRANÇAISE  
DE THROMBOSE  
ET D'HÉMOSTASE

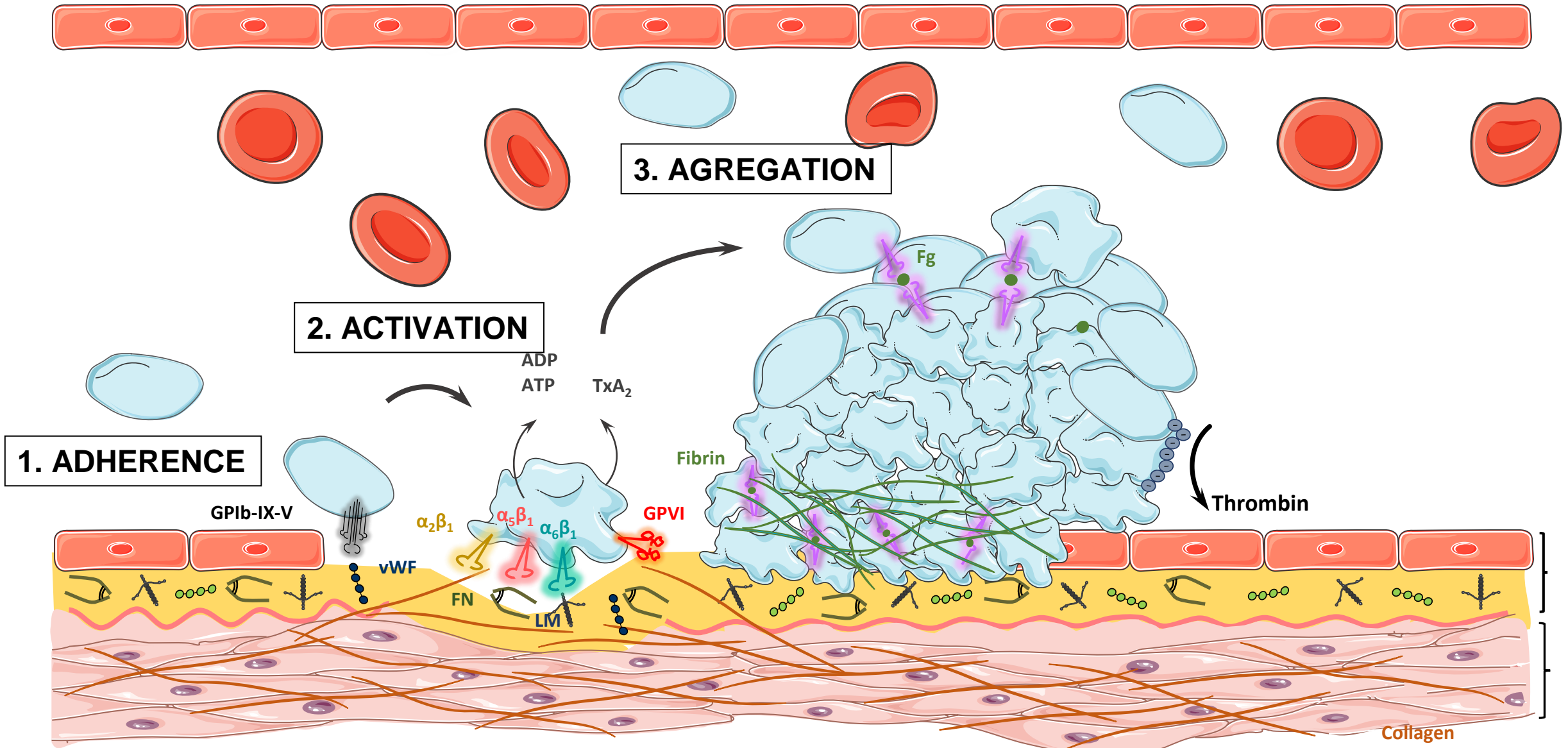


Pierre Mangin  
Inserm U1255  
Université de Strasbourg

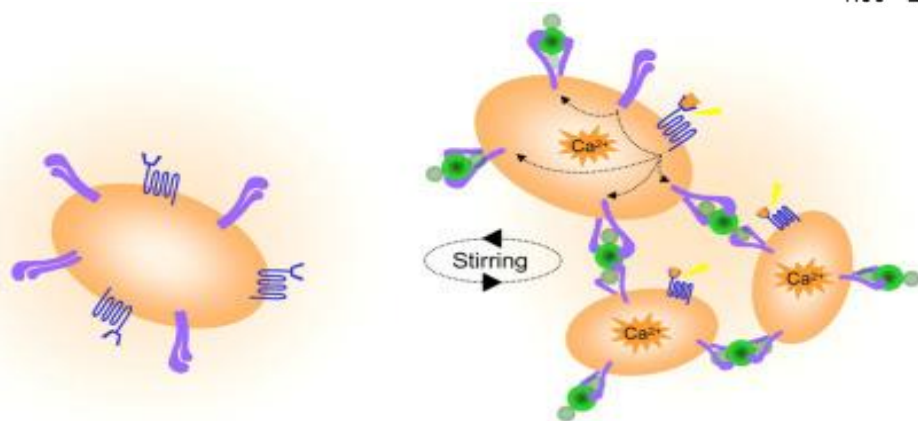
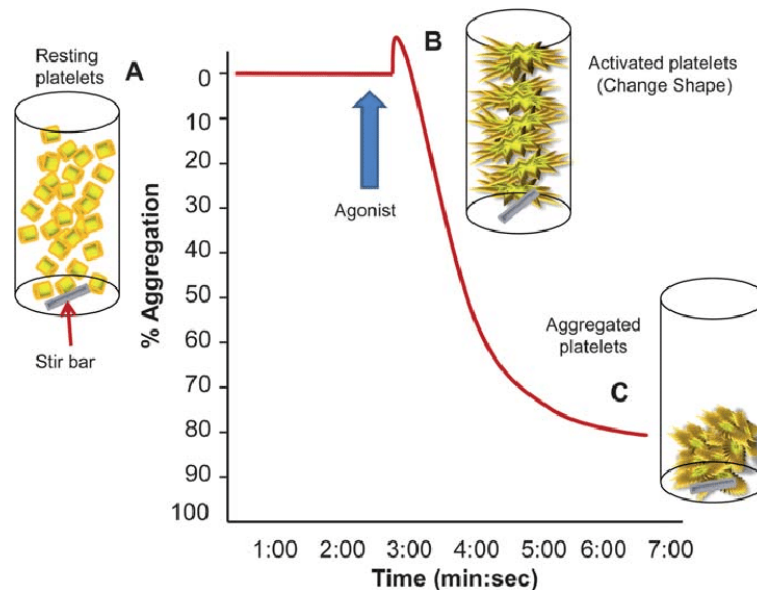
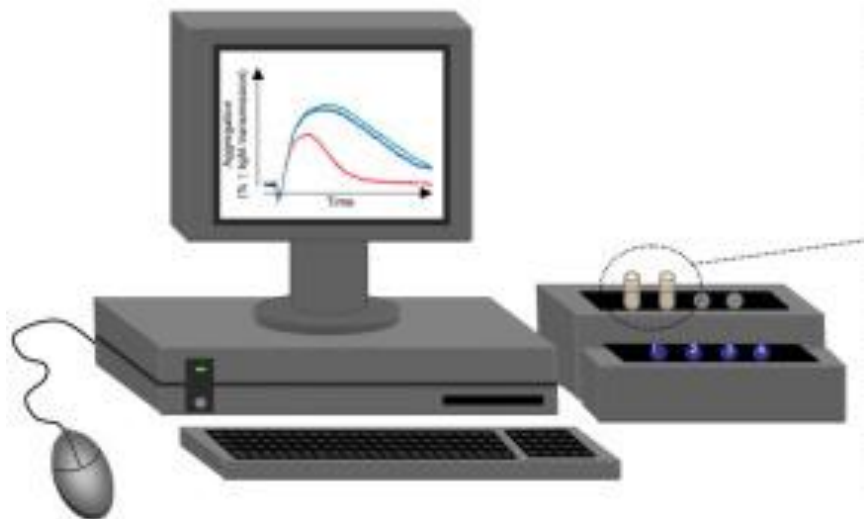


## MODELES D'ETUDE DE L'HEMOSTASE ET DE LA THROMBOSE EN FLUX

# L'hémostase



# L'agrégomètre de Gustave Born: des avancées majeures dans la compréhension de la physiologie plaquettaire

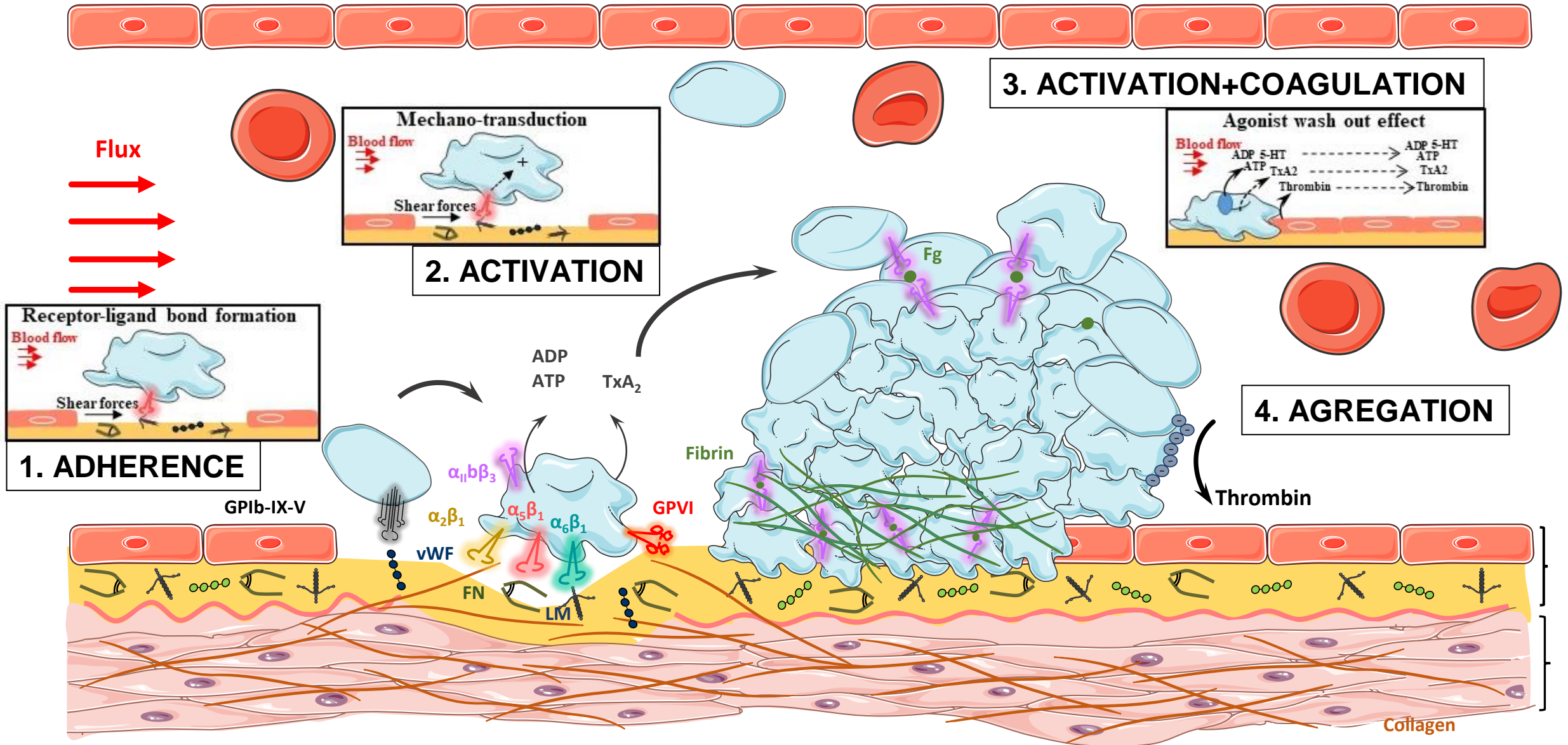


**Key**

- Inactive/active  $\alpha_2\beta_3$
- GPCR
- Fibrinogen
- Thrombin
- Inside-out signalling
- $Ca^{2+}$  Calcium mobilisation
- ADP/TxA2

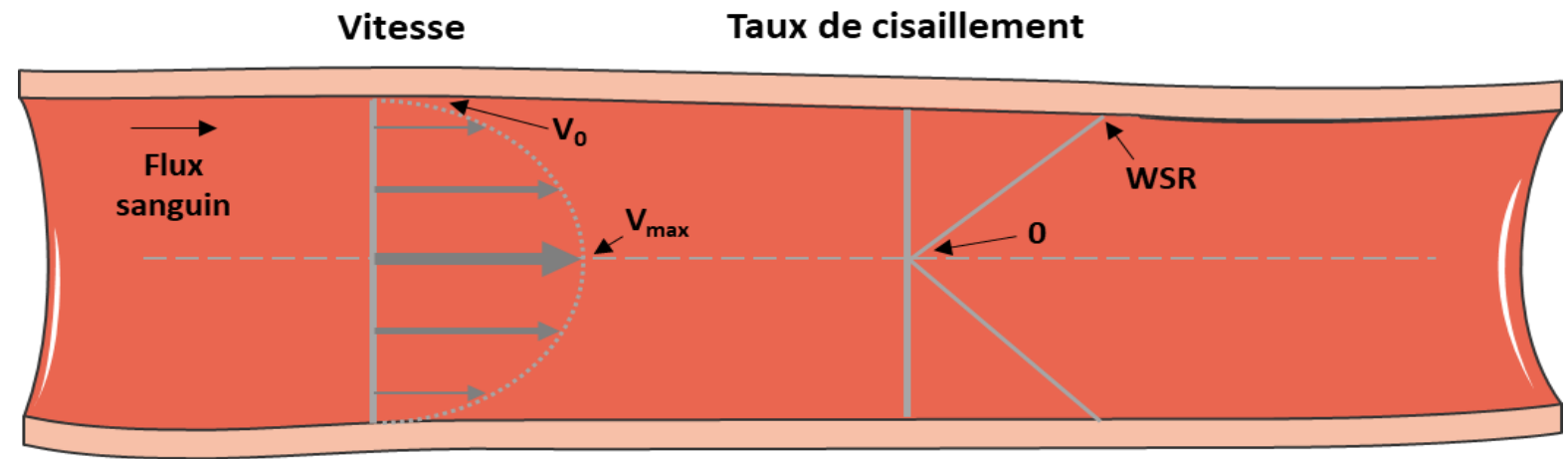


# L'importance du flux dans le processus de l'hémostase



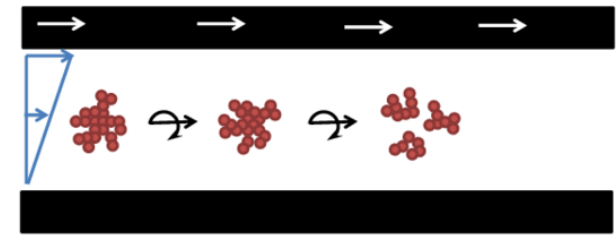
# Notions de base d'hémodynamique

**La loi de Poiseuille** décrit l'écoulement laminaire d'un liquide visqueux incompressible dans un cylindre



**Le taux de cisaillement ( $\gamma$ )** est un gradient de vitesse perpendiculaire à la **direction** du flux ( $s^{-1}$ )

$$\gamma = \frac{4Q}{\pi R^3}$$



# Quelles sont les conditions d'écoulement dans le corps ?

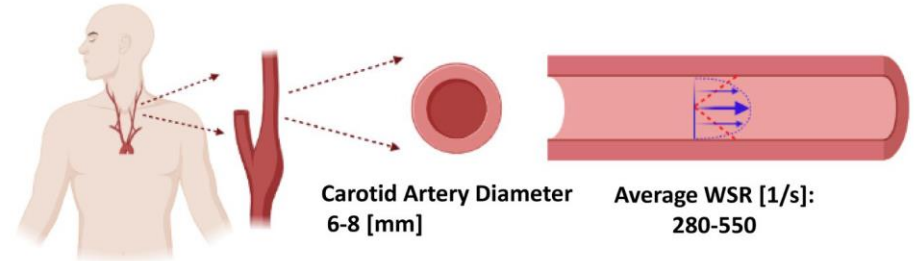
## RECOMMENDATIONS AND GUIDELINES

jth

### Wall shear rates in human and mouse arteries: Standardization of hemodynamics for in vitro blood flow assays: Communication from the ISTH SSC subcommittee on biorheology

Mikhail A. Pantelev<sup>1,2</sup> | Netanel Korin<sup>3</sup> | Koen D. Reesink<sup>4</sup> | David L. Bark<sup>5</sup> |  
Judith M. E. M. Cosemans<sup>6</sup> | Elizabeth E. Gardiner<sup>7</sup> | Pierre H. Mangin<sup>8</sup>

Human male average height [cm] : 170



Mouse average length [cm] : 10

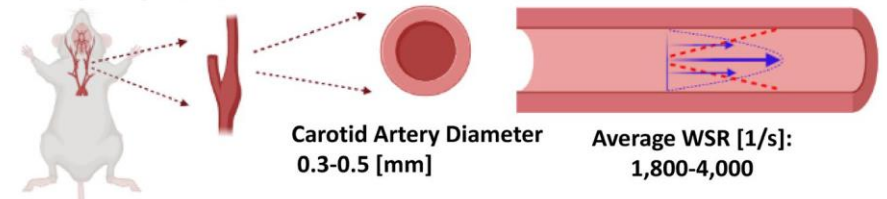
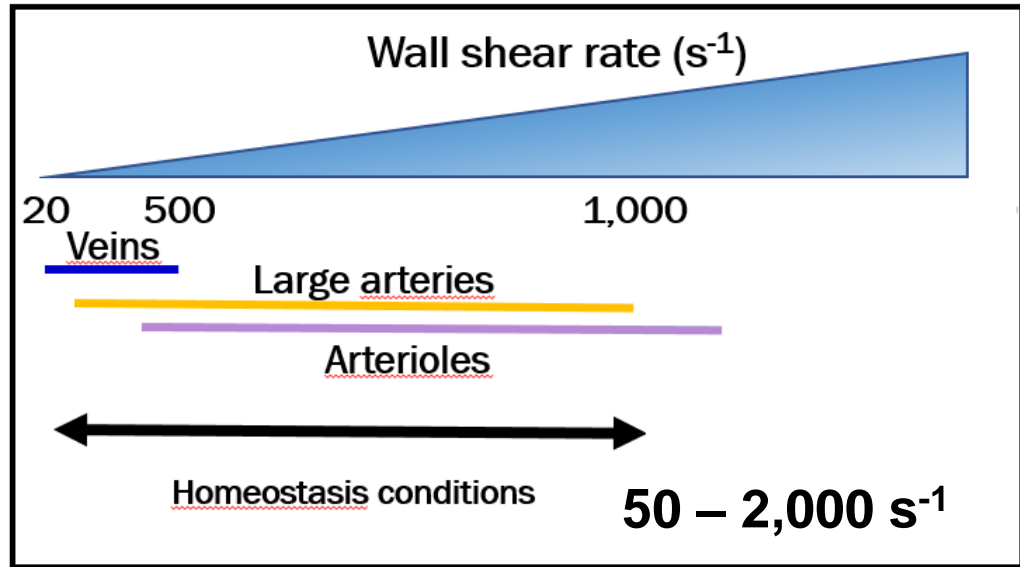
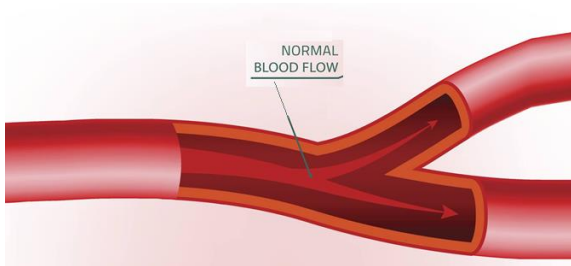


TABLE 1 Vessel wall shear rates in human and murine non-pathological blood vessels

Human vessel	Diameter, mm	Average wall shear rate, s <sup>-1</sup>	Murine vessel (experimental model (s) of thrombosis)	Diameter, μm	Average wall shear rate, s <sup>-1</sup>
Carotid artery	4.3--8.2 <sup>16</sup>	260--500 <sup>16</sup>	Carotid artery (ferric-chloride-induced thrombosis)	300--500 <sup>17</sup>	1800--4000 <sup>17-19,45</sup>
Coronary artery	2.6--5.0 <sup>13</sup>	100--350 <sup>13</sup>	Coronary artery (histology in ApoE <sup>-/-</sup> mice)	230 <sup>23</sup>	6700 <sup>23</sup>
Aorta	14.0--20.0 <sup>17</sup>	40--150 <sup>17</sup>	Aorta (mechanical injury)	700 <sup>10</sup>	2500 <sup>10</sup>
Mesenteric arteries	0.41--0.66 <sup>26</sup>	170--330 <sup>26</sup>	Mesenteric arteries (laser-induced, ferric-chloride-induced)	80--150 <sup>27,28</sup>	1200--1700 <sup>27,28</sup>
Cremaster arteriole			Cremaster arteriole (laser-induced)	40--60 <sup>30,31</sup>	700--1000 <sup>30,31</sup>

# Quelles sont les conditions d'écoulement après une lésion vasculaire?

## Conditions physiologiques



## Scénarios de l'hémostase

### Ponction

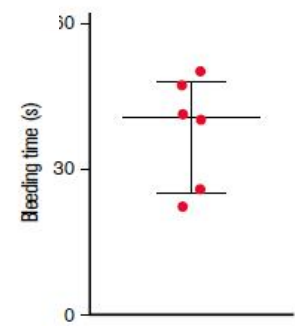
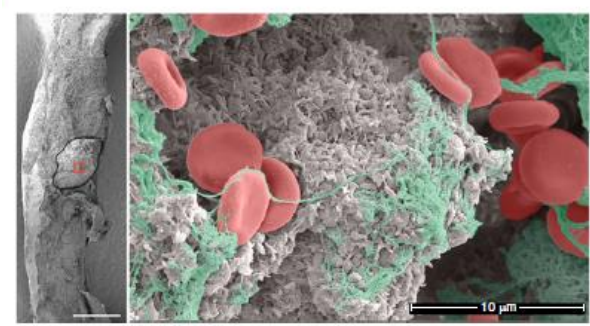
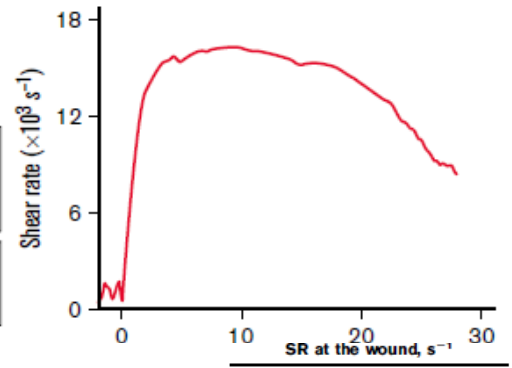
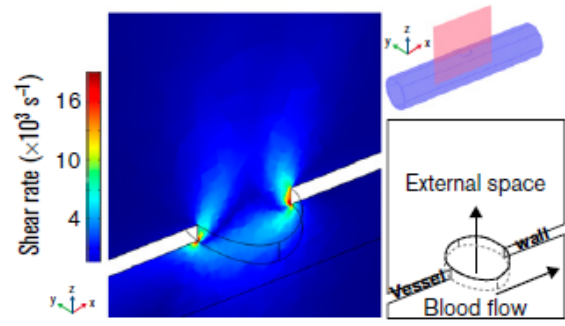
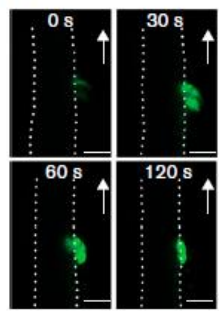
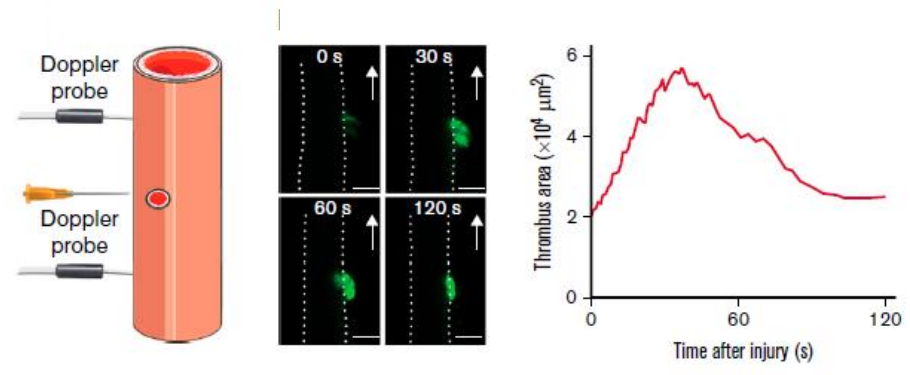


### Rupture



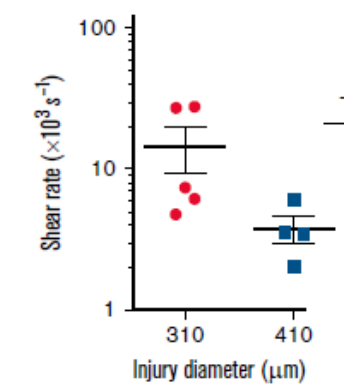
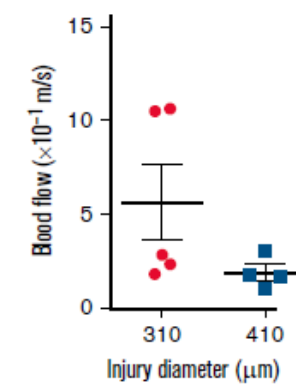
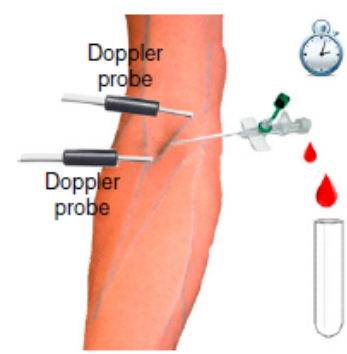
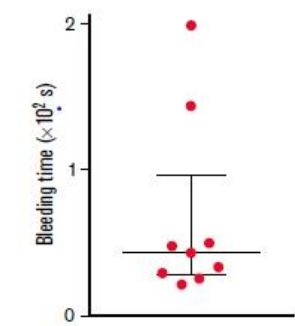
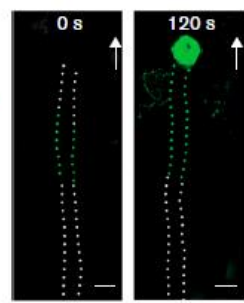
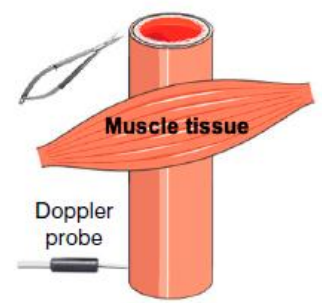
Question: quelles sont les conditions d'écoulement après une lésion vasculaire ?

# Quelles sont les conditions d'écoulement après une lésion vasculaire?



Vessel	Species	Type of vessel injury	Diameter of the injury, $\mu\text{m}$	Median	25%-75% percentile
Carotid artery	Mouse	Puncture	108-280	22000	5600-85000
Aorta	Mouse	Puncture	40-240	25000	6000-138000
Saphenous vein	Mouse	Puncture	66-173	7000	3000-31000
Spermatic artery	Mouse	Disruption	215	14500	11000-20000
Median cubital vein	Man	Puncture	310	7400	5400-27300
			410	3500	2300-5400

SR, shear rate.





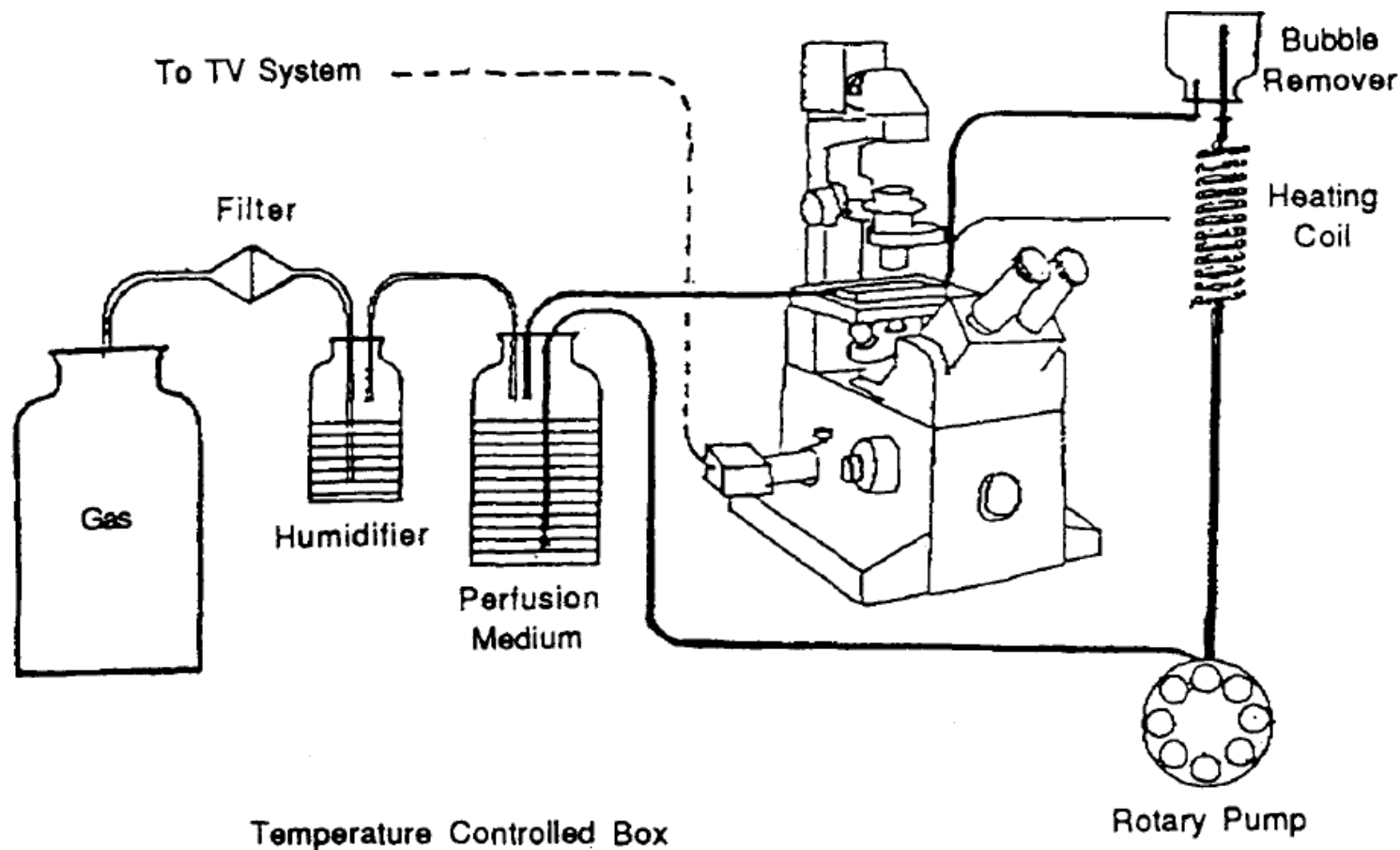
# Les systèmes de perfusion: de la genèse aux systèmes classiques

# Premiers systèmes de perfusion : « parallel plate »

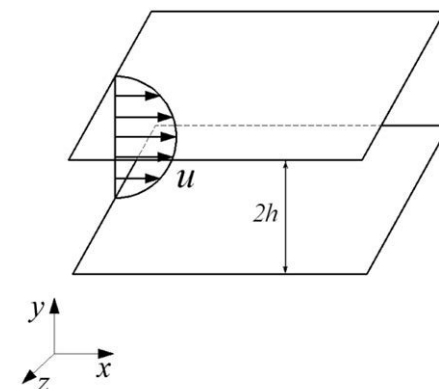
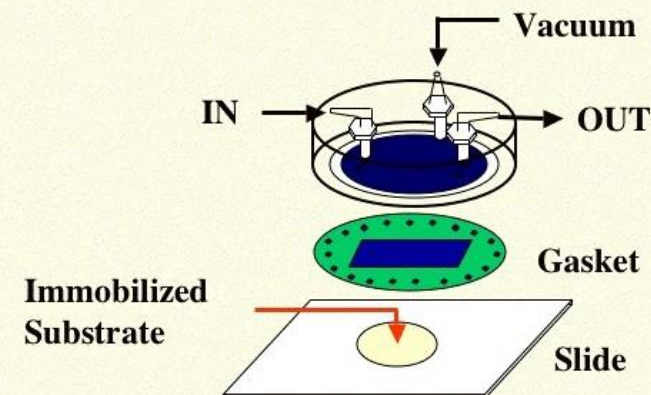
## Design and Construction of a Linear Shear Stress Flow Chamber

SHUNICHI USAMI,\* HSUAN-HSU CHEN,\* YIHUA ZHAO,† SHU CHIEN,† and RICHARD SKALAK†

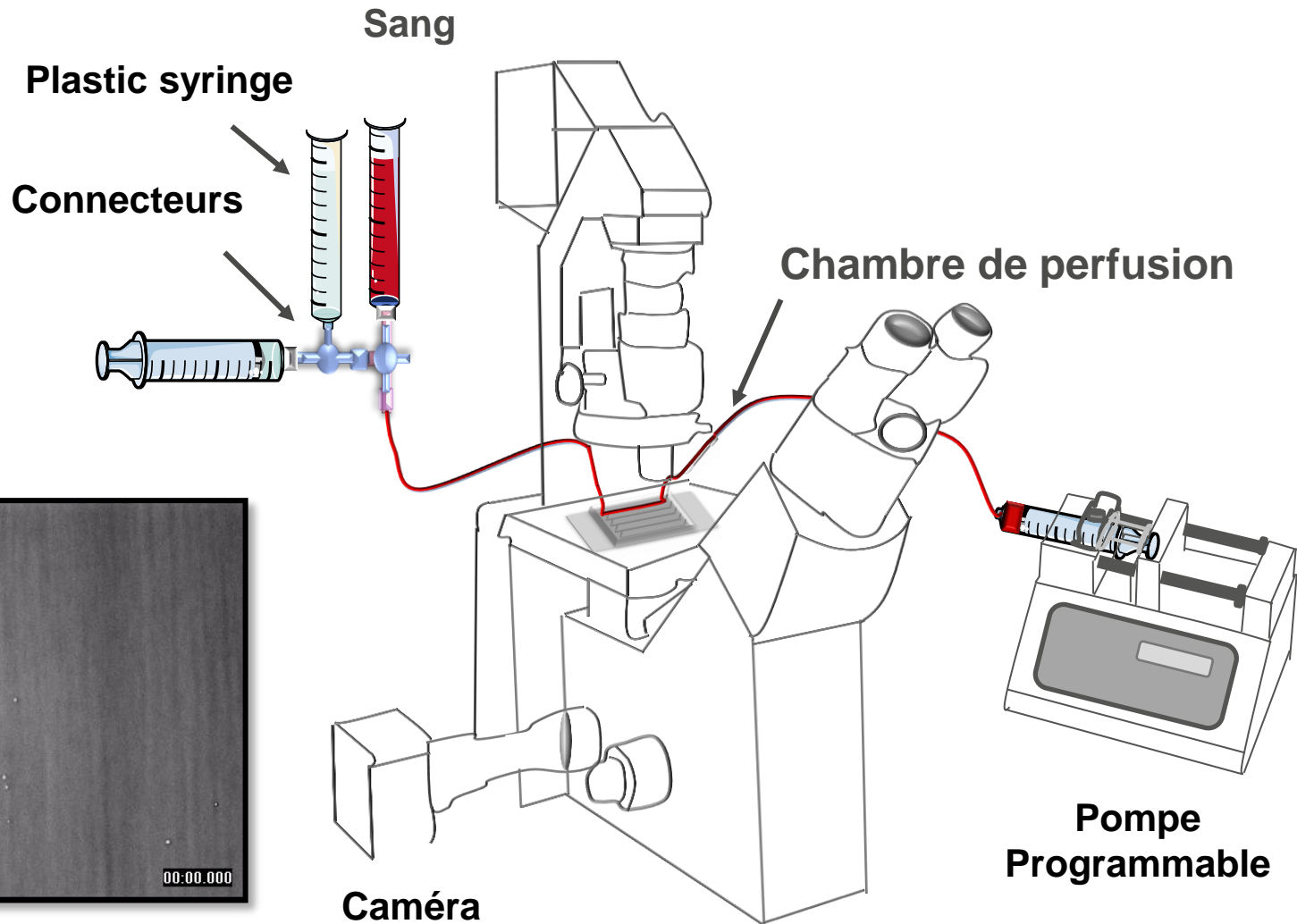
\*Institute of Biomedical Sciences, Academia Sinica, Taipei, 11529, Taiwan,  
†Department of Applied Mechanics and Engineering Sciences/Bioengineering,  
University of California, San Diego, La Jolla, CA



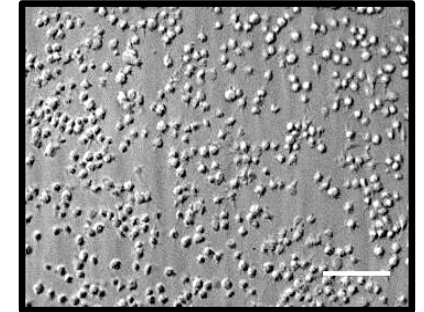
## Parallel Plate Flow Chamber Assay



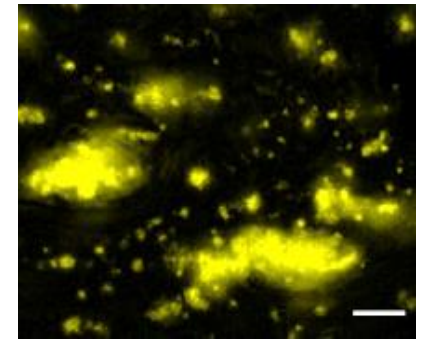
# Systeme de perfusion pour étudier les fonctions des plaquettes



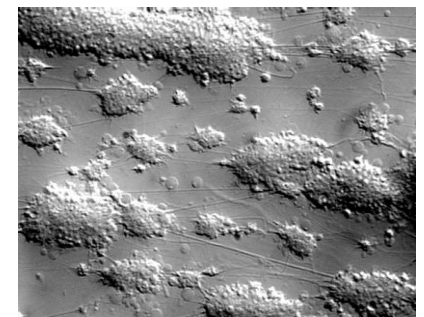
Adhérence



Activation



Agrégation

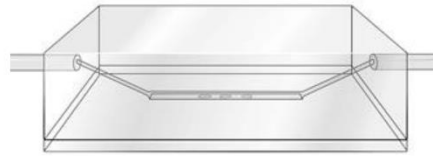


# Systeme de perfusion pour étudier les fonctions des plaquettes

## Capillaires de verre



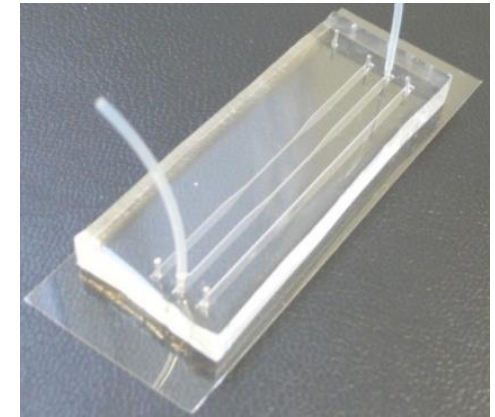
## Chambre en flux de Maastricht



## Chambres commerciales



## Chambres microfluidiques



**Faible volume sanguin**  
Disponible dans le commerce  
Peu couteux

**Faible volume sanguin**  
Bien caractérisée

**Faible volume sanguin**  
Disponible dans le commerce  
Facile d'usage  
Compatible avec la culture cell.

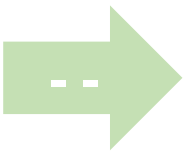
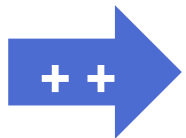
**Faible volume sanguin**  
Facile d'usage  
Tout type de géométrie  
Peu Couteux

Géométrie simple  
Délicat d'usage

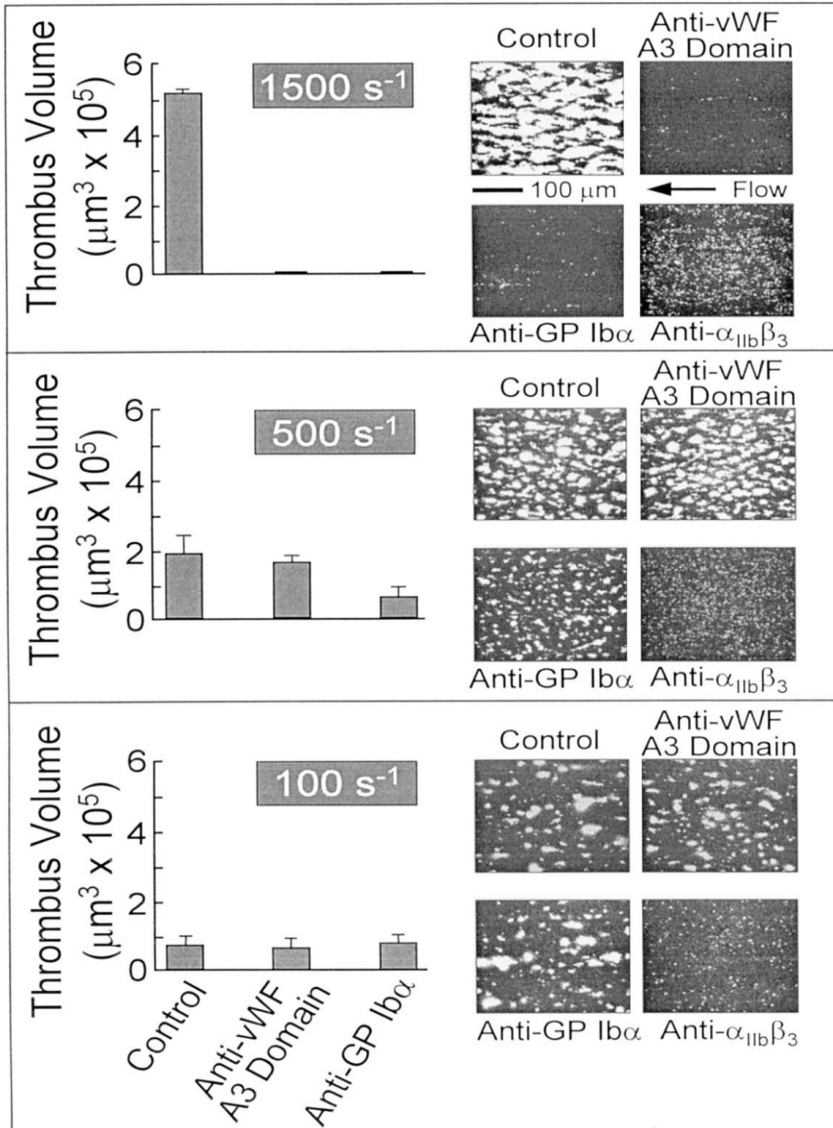
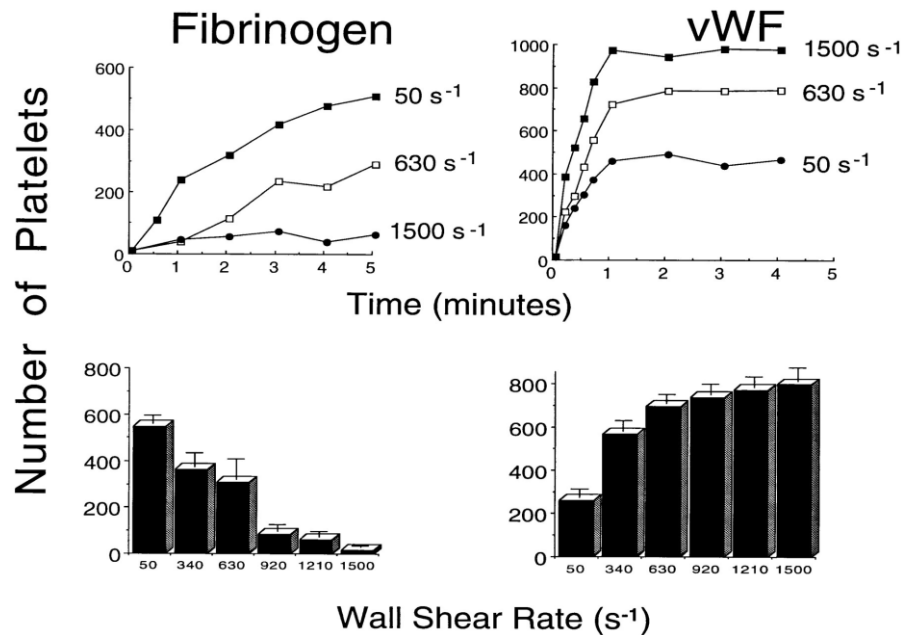
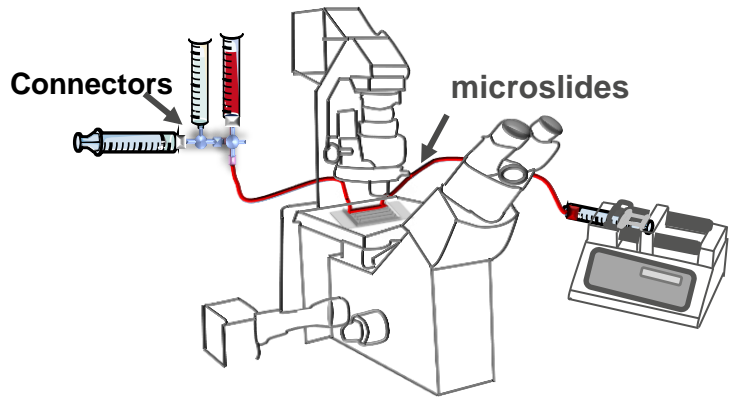
Géométrie simple  
Fait maison

Géométrie simple  
Couteux  
Nécessite un équipement spécialisé

?



# Avancées majeures dans la compréhension des mécanismes moléculaires de l'hémostase primaire



**Z. Ruggeri**

 Scripps Research

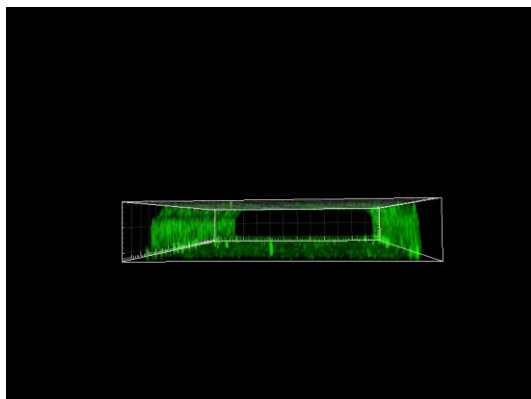
*Savage et al., Cell, 1996*  
*Savage et al., Cell, 1998*

# Les systèmes de perfusion: Se rapprocher de l'hémostase

# Systèmes de perfusion pour l'étude de l'interaction sang/endothélium

## Assessment of whole blood thrombosis in a microfluidic device lined by fixed human endothelium

Abhishek Jain<sup>1,2,3</sup> · Andries D. van der Meer<sup>1,4</sup> · Anne-Laure Papa<sup>1</sup> · Riccardo Barrile<sup>1,5</sup> · Angela Lai<sup>6</sup> · Benjamin L. Schlechter<sup>7</sup> · Monicah A. Otieno<sup>8</sup> · Calvert S. Loudon<sup>8</sup> · Geraldine A. Hamilton<sup>1,9</sup> · Alan D. Michelson<sup>10</sup> · Andrew L. Frelinger III<sup>10</sup> · Donald E. Ingber<sup>1,3,11</sup>

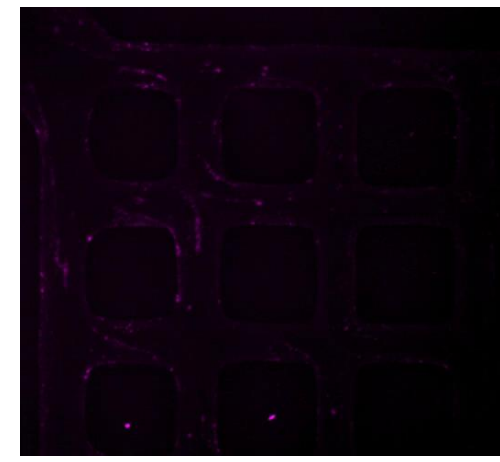
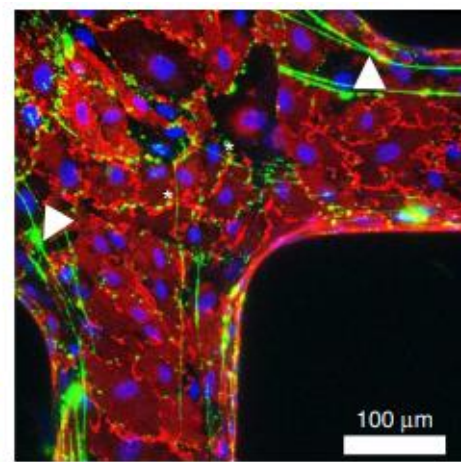
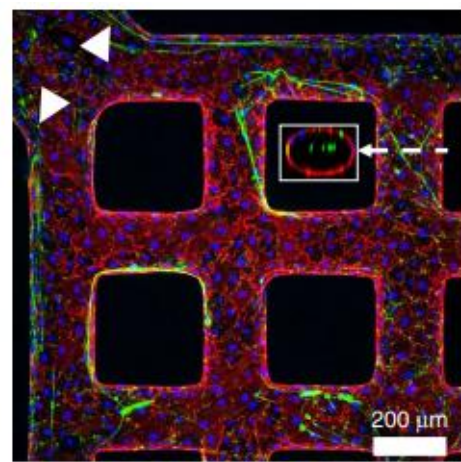
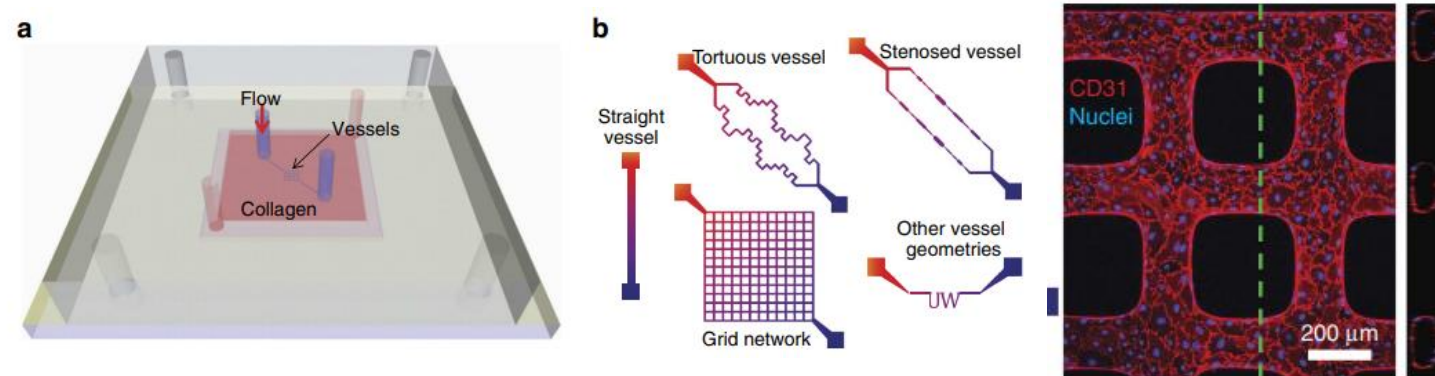


Chemopreserved Endothelium

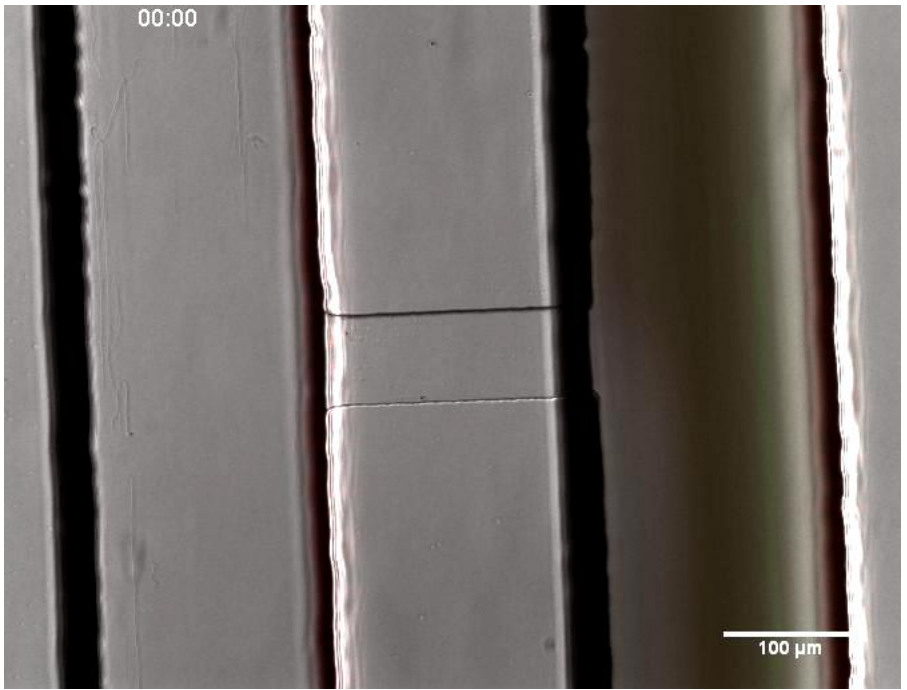
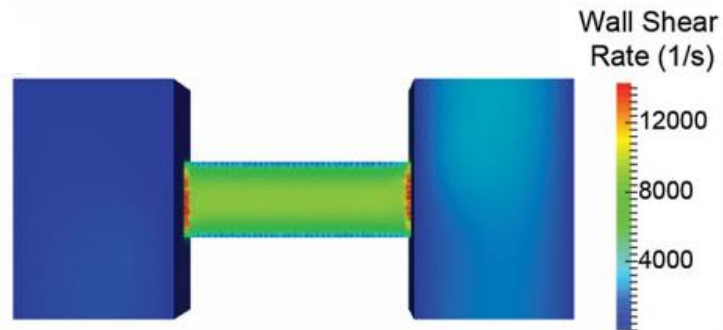
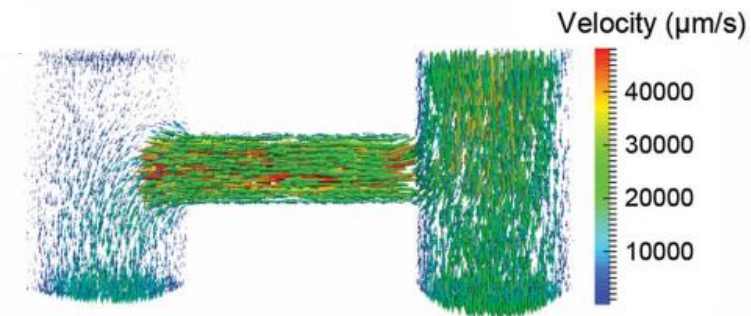
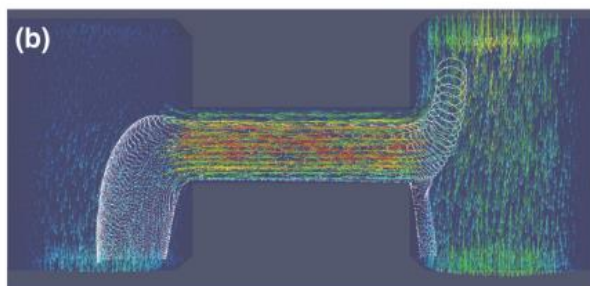
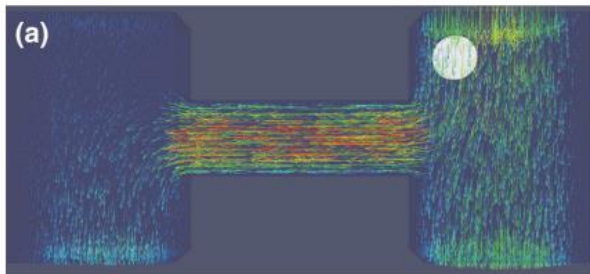
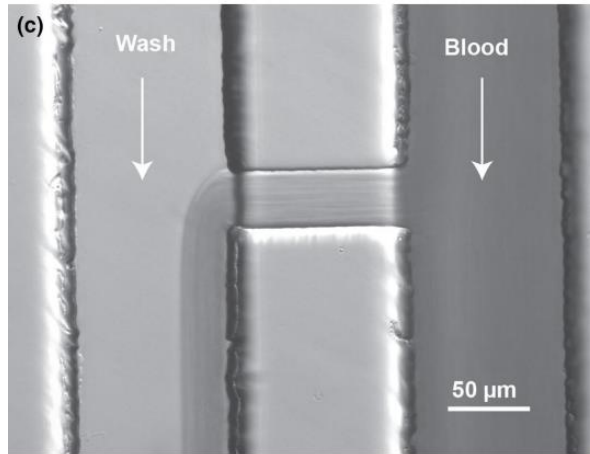


## Flow-driven assembly of VWF fibres and webs in *in vitro* microvessels

Ying Zheng<sup>1,2</sup>, Junmei Chen<sup>3</sup> & José A. López<sup>3,4</sup>



# Système en flux qui mime une lésion vasculaire

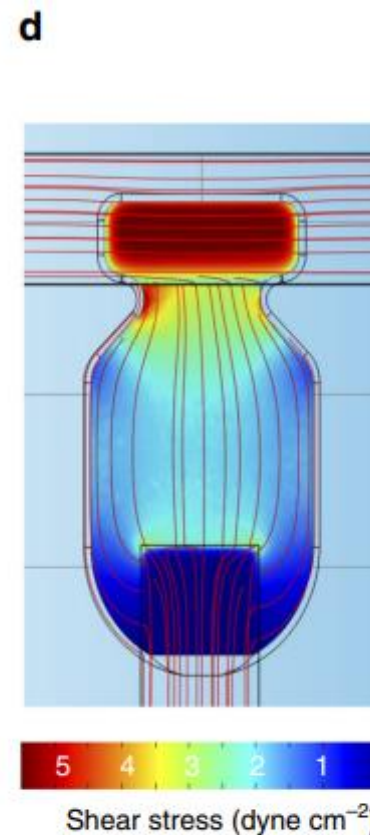
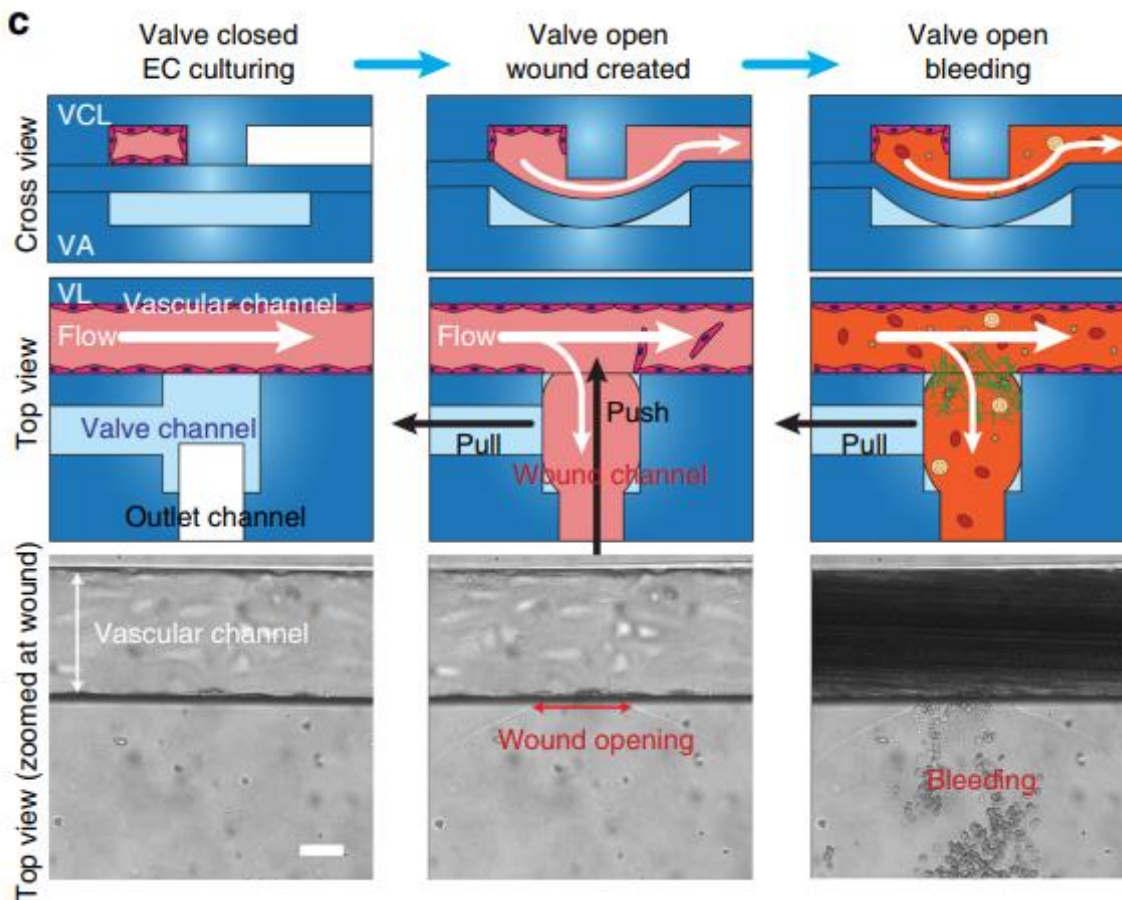
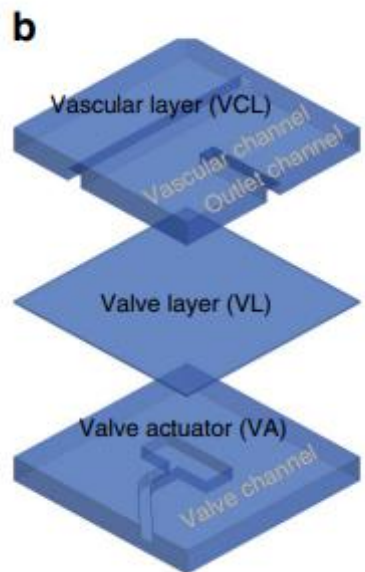




# Système en flux qui mime une lésion vasculaire

nature  
COMMUNICATIONS

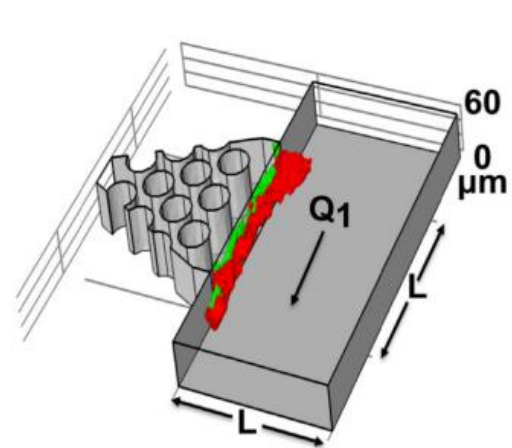
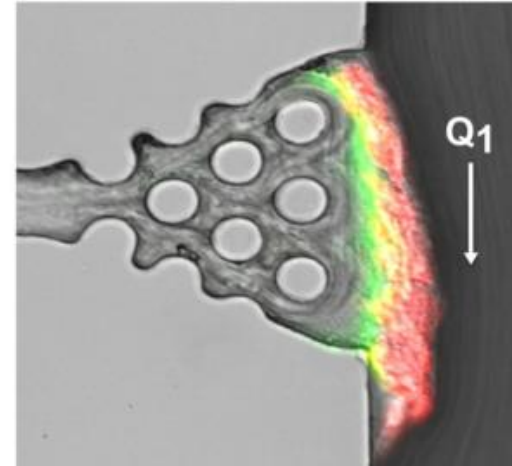
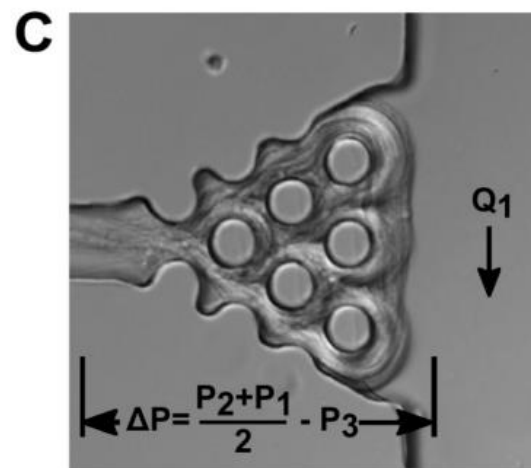
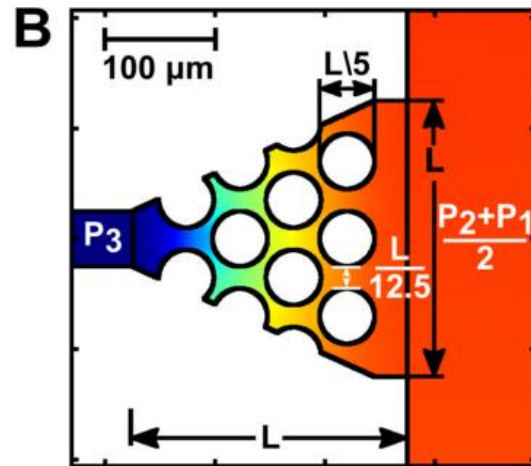
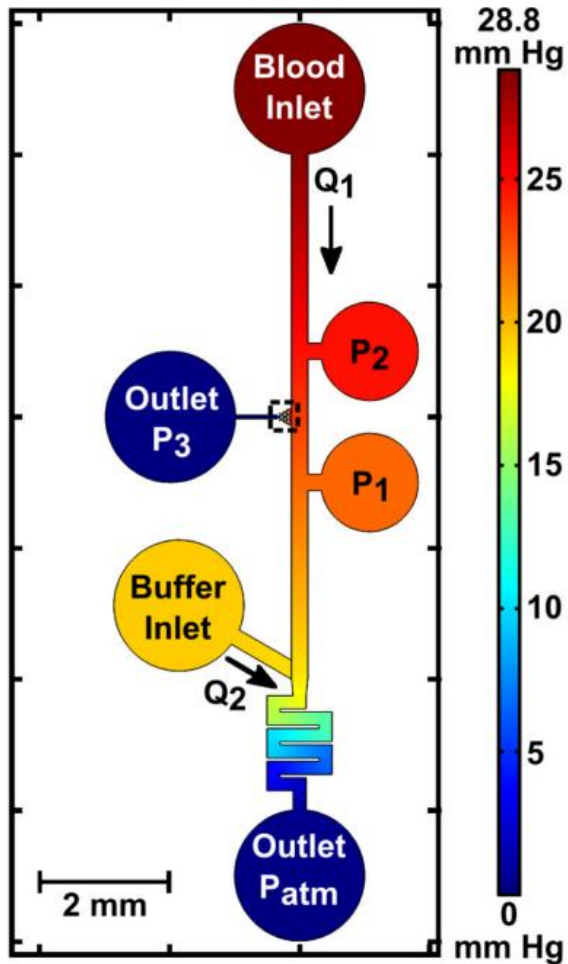
A microengineered vascularized bleeding model that integrates the principal components of hemostasis



W. Lam

EMORY  
WINSHIP  
CANCER  
INSTITUTE  
National Cancer Institute-Designated  
Comprehensive Cancer Center

# Systeme en flux qui mime une lésion vasculaire

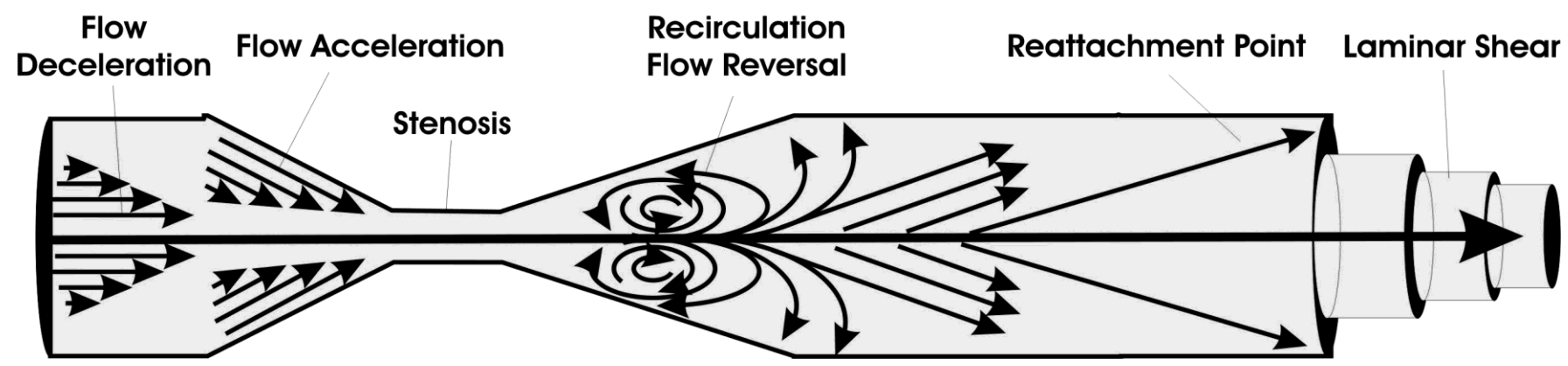
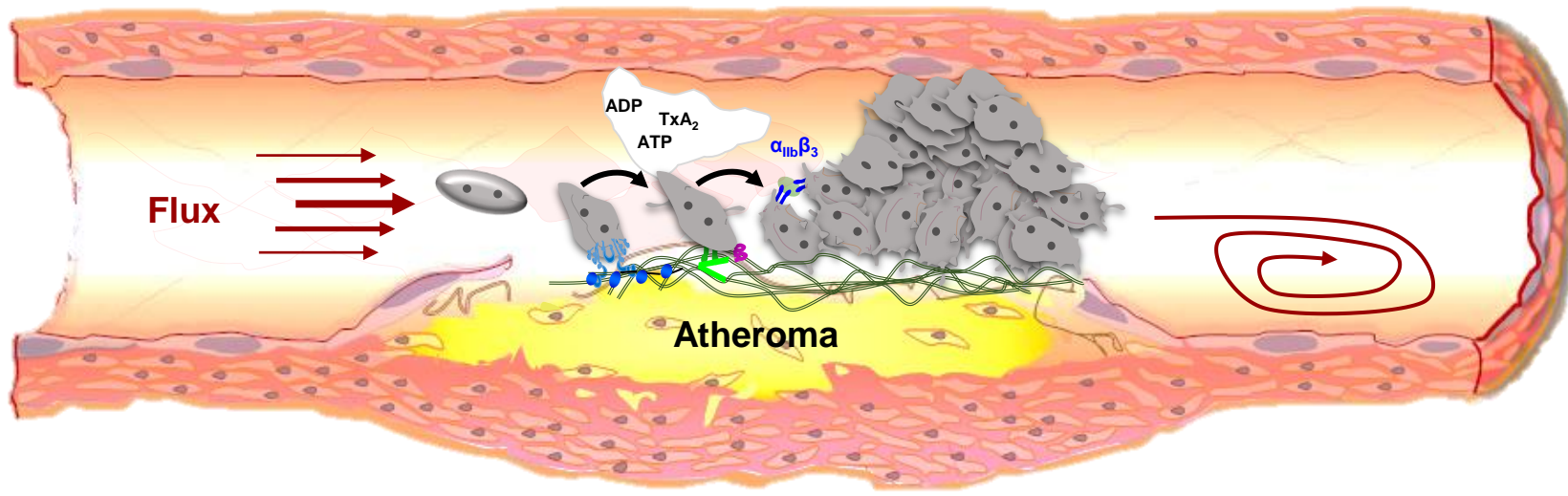


**S. Diamond**  
 Penn Engineering  
 UNIVERSITY OF PENNSYLVANIA

Muthard *et al.*, *ATVB*, 2012  
 Herbig *et al.*, *Cell Mol Bioeng*, 2017

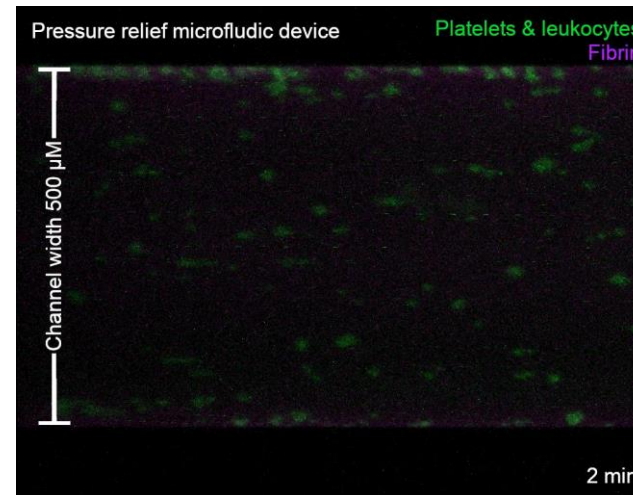
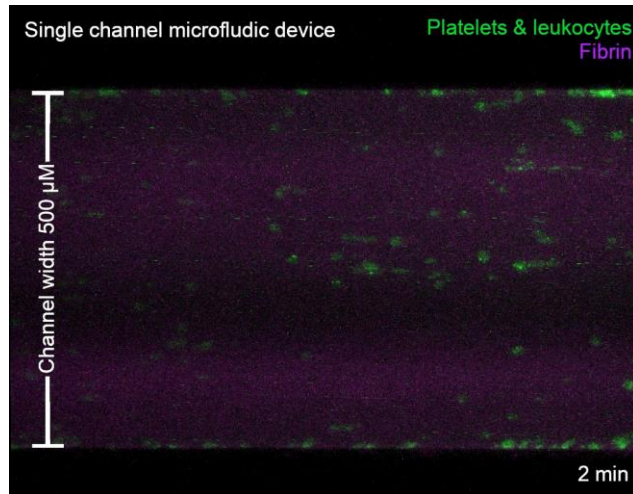
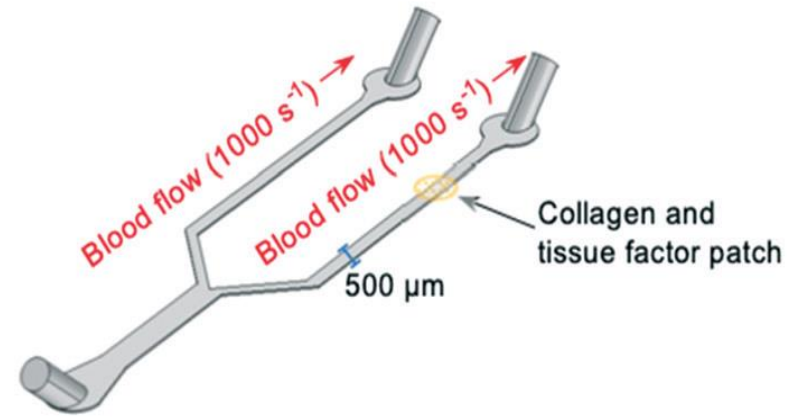
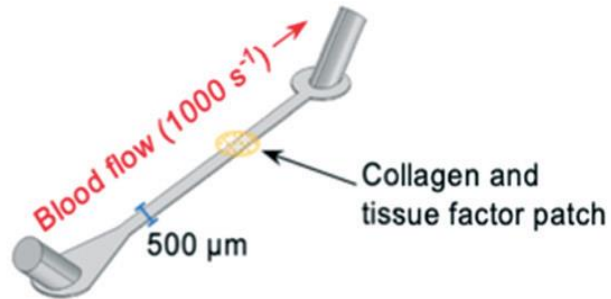
# Les systèmes de perfusion: Se rapprocher de la thrombose artérielle

# L'importance du flux dans la thrombose artérielle



# Modèle de perfusion pour générer un thrombus occlusif

## Thrombose occlusive



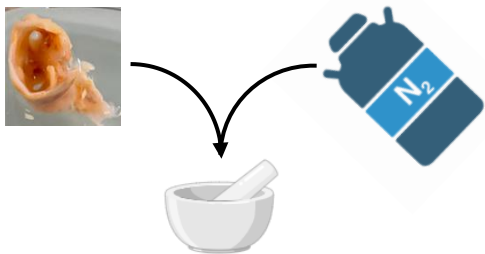
M. Harper



UNIVERSITY OF  
CAMBRIDGE

# Modèle de perfusion pour générer un thrombus occlusif

Cryobroyage à l'azote  
liquide d'une plaque entière



Obtention d'une  
poudre fine



Homogénats



Reprise en PBS

Centrifugation

Récupération de la couche  
homogène du milieu



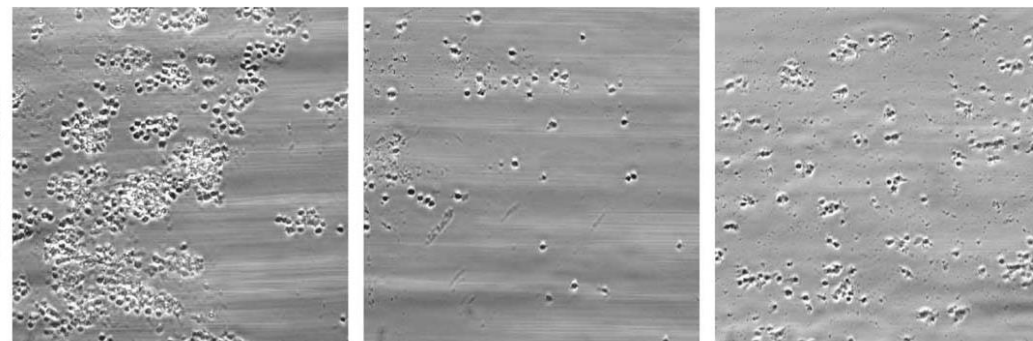
Surnageant  
Protéines  
Débris

Control

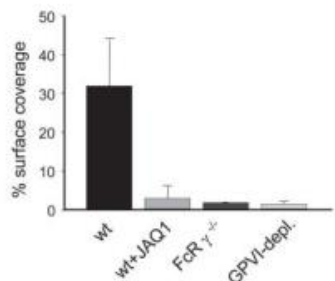
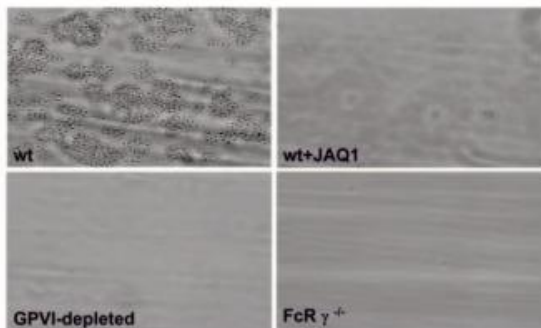
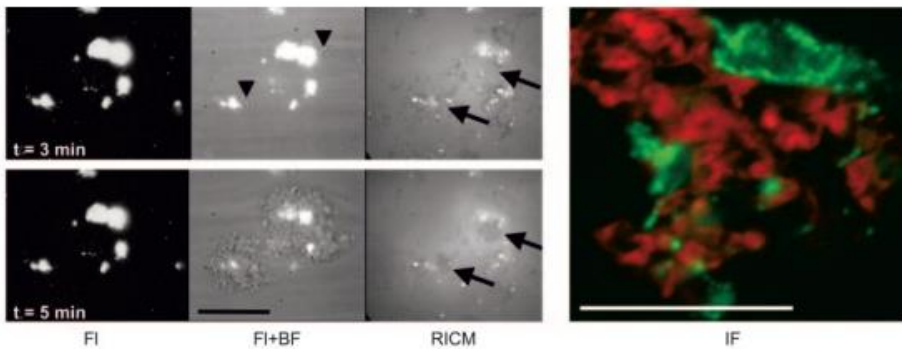
$\alpha$ -GPVI

P2Y block

Phase contrast

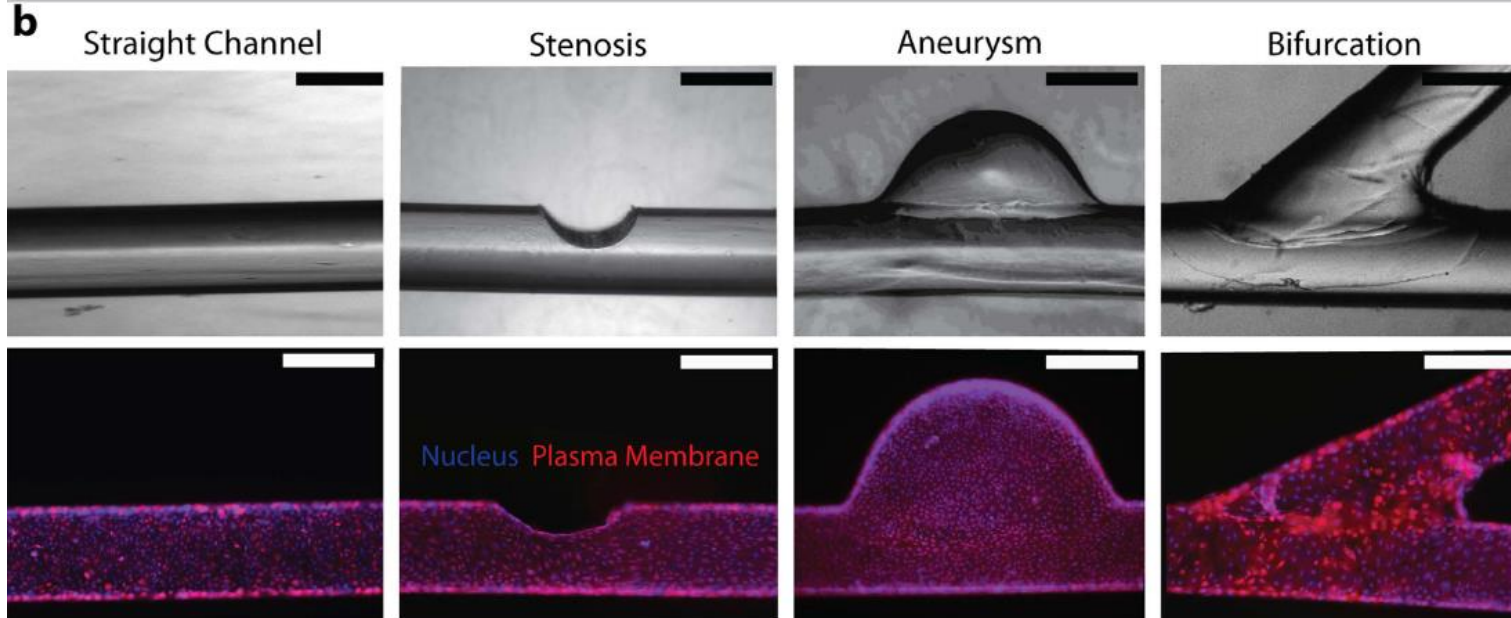
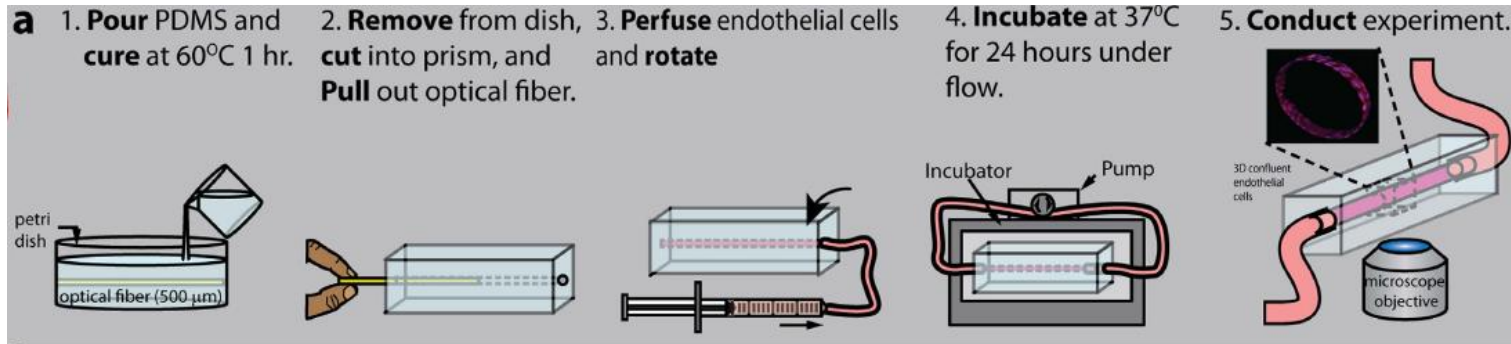


OG488 annexin A5



# Systemes en flux qui se rapprochent de l'anatomie humaine

“Do-it-yourself *in vitro* vasculature that recapitulates *in vivo* geometries for investigating endothelial-blood cell interactions”



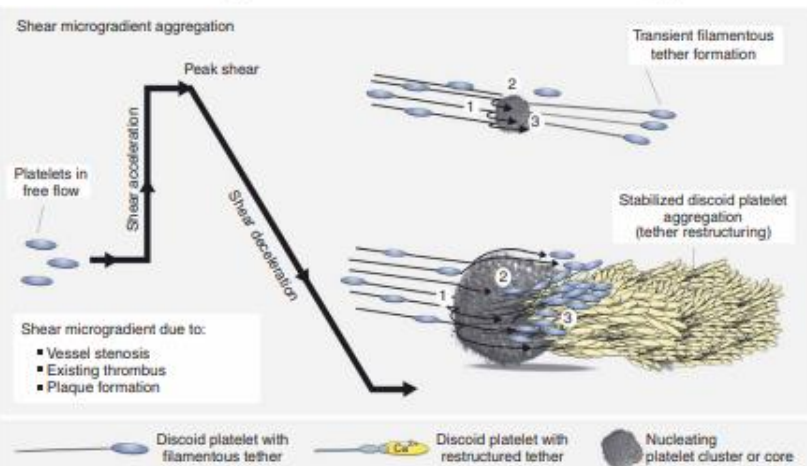
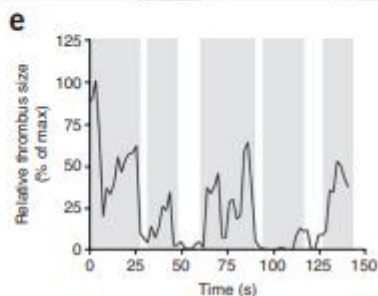
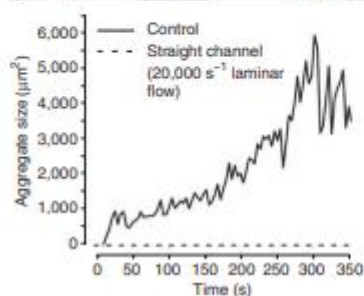
W. Lam

EMORY  
WINSHIP  
CANCER  
INSTITUTE  
National Cancer Institute-Designated  
Comprehensive Cancer Center

# Une chambre qui mime une sténose artérielle

**nature  
 medicine**

A shear gradient-dependent platelet aggregation mechanism drives thrombus formation



Supplementary Video 2

Shear Micro-gradient Induced Platelet Aggregation *In Vitro*

PDMS microchannels

SP Jackson

HEART RESEARCH INSTITUTE

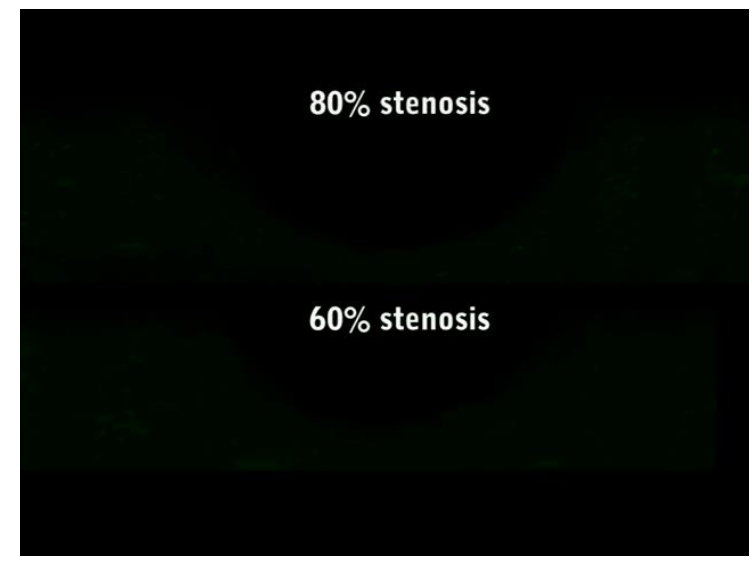
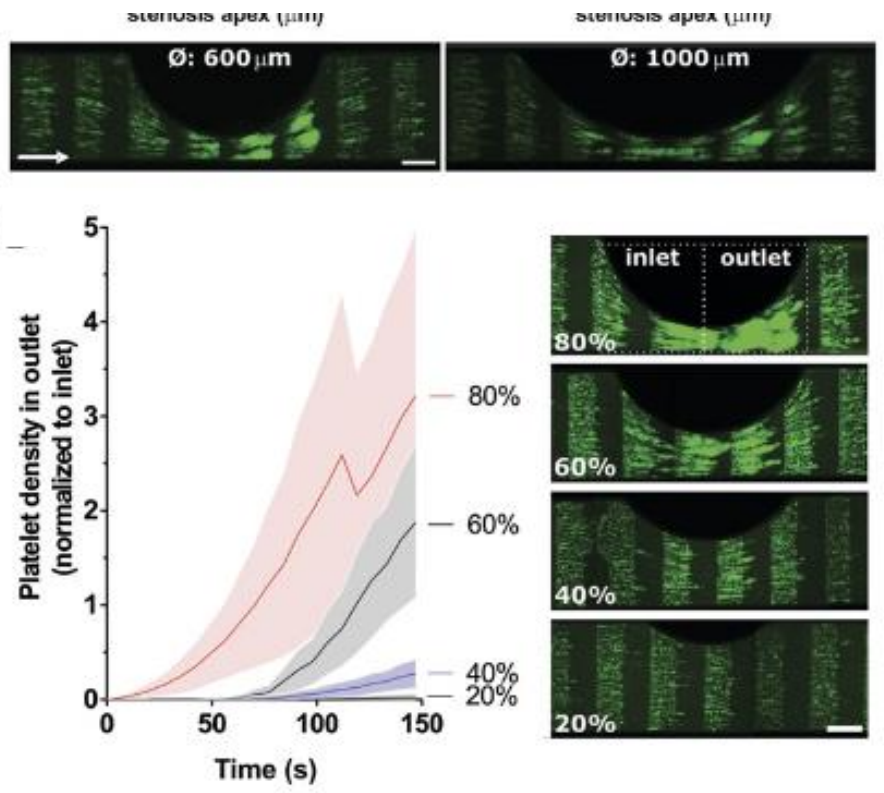
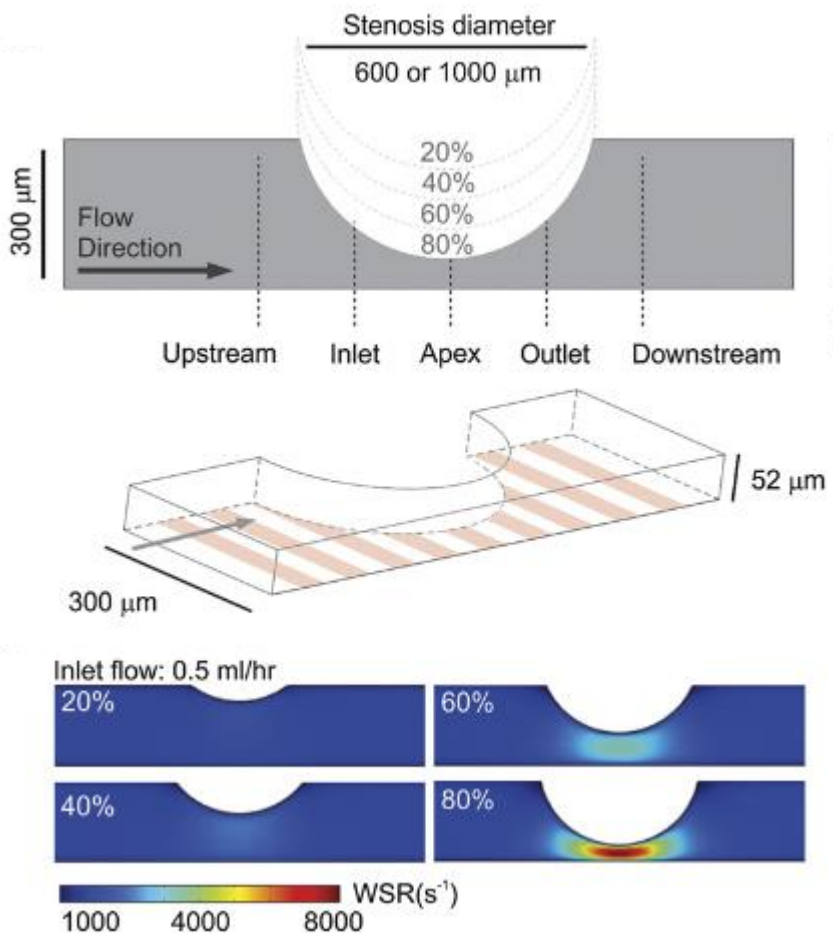
Nesbitt *et al.*, *Nature medicine*, 2009



# Une chambre qui mime une sténose artérielle

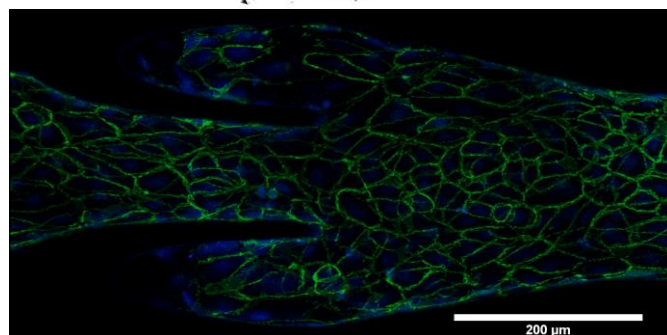
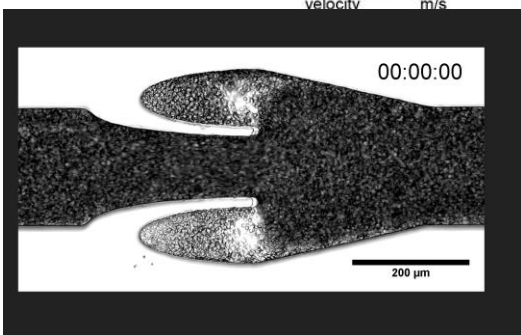
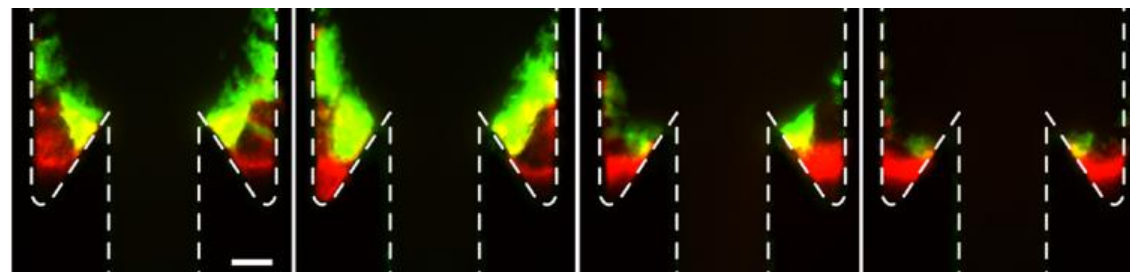
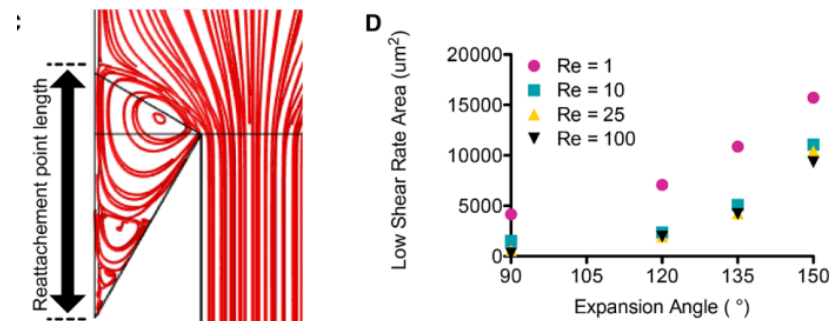
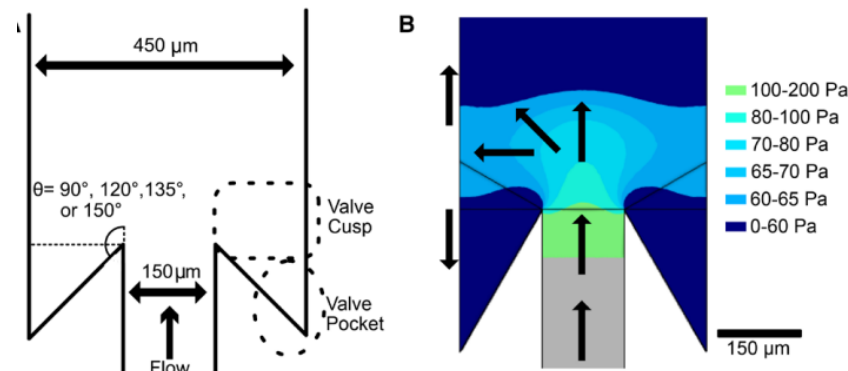
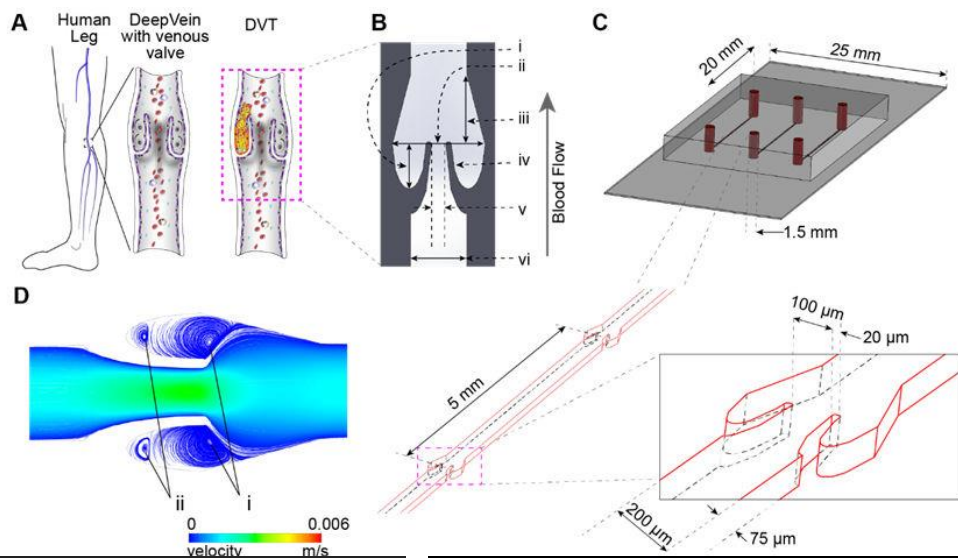
**PNAS** Proceedings of the National Academy of Sciences of the United States of America

**Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner**



# Systemes de perfusion pour étudier la thrombose veineuse

## Microengineered Human Vein-Chip Recreates Venous Valve Architecture and Its Contribution to Thrombosis

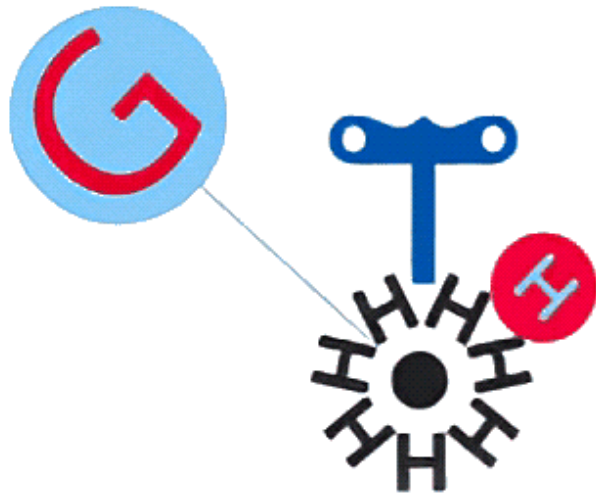


# Conclusion-discussion

- **L'hémodynamique joue un rôle majeur dans la fonction des plaquettes, dans l'hémostase et dans la thrombose artérielle**
- **Les dispositifs en flux « classiques » ont permis des avancées majeures dans l'étude des fonctions plaquettaires et restent très utilisés en recherche: On ne reproduit ni la structure du thrombus, ni la dynamique**
- **Développement de nombreux biomimétiques: quelle est la valeur ajoutée ?**
- **Des dispositifs en flux sont également utilisés comme outil diagnostique: T-Tas**
- **Que manque-t-il et quelles sont les prochaines étapes ?**
  - **Complexité à inclure la coagulation dans les systèmes en flux**
  - **Modèles de l'AVC et de la thrombo-inflammation**
  - **Aucun dispositif ne permet d'étudier le risque hémorragique**



# Society of Thrombosis and Haemostasis Research



**GTH 2025 / ARTE in Lausanne**  
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## Save the date

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Advances  
Research  
Technology  
Education

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# Acknowledgements



## **INSERM 1255:**

**Alexandra Yakusheva**

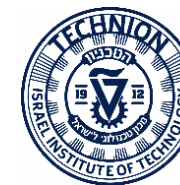
**Catherine Bourdon**

**Clarisse Mouriaux**

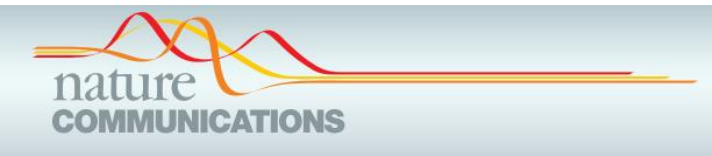
**Lise Charles**

**Miao Feng**

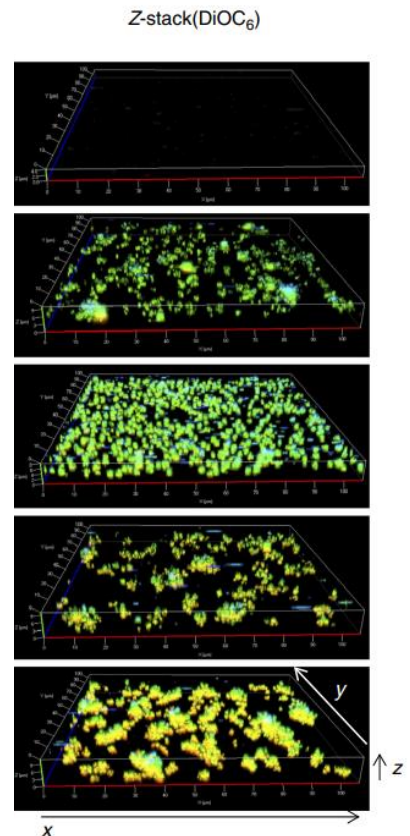
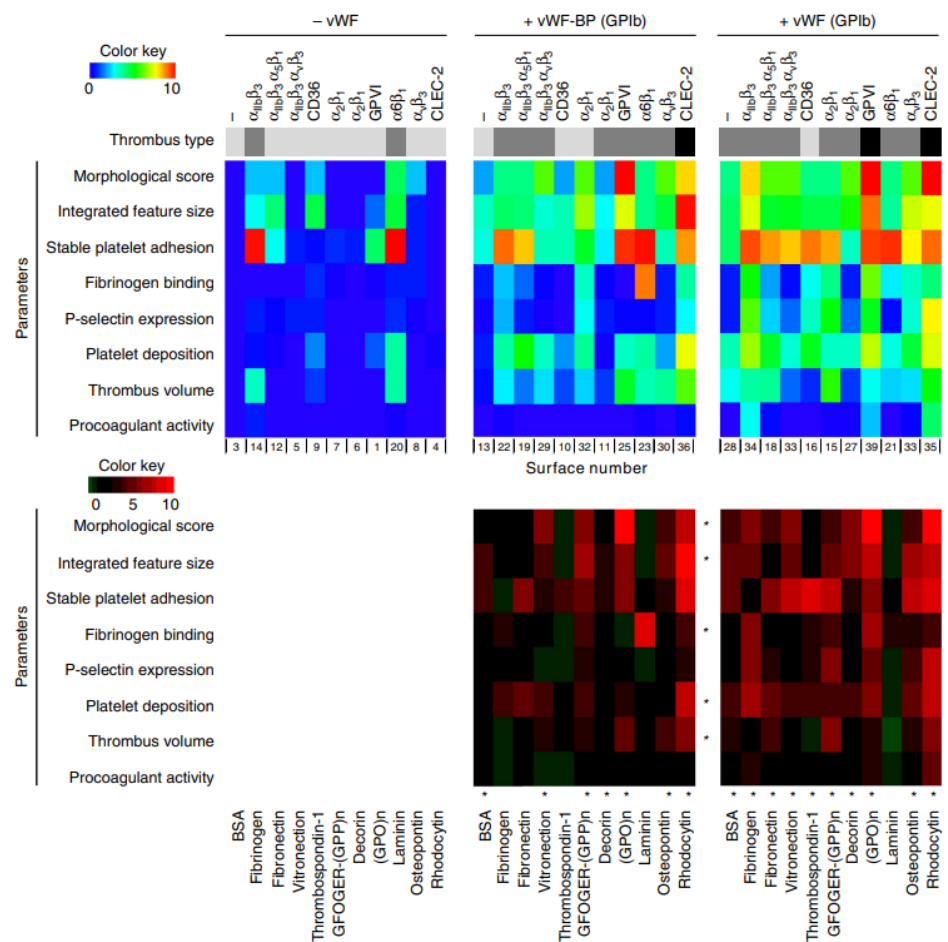
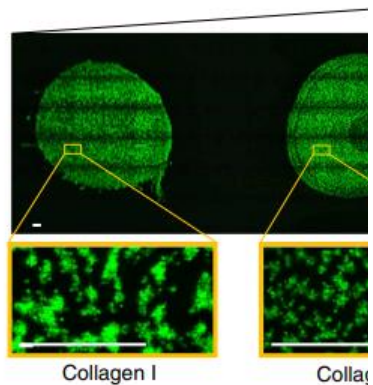
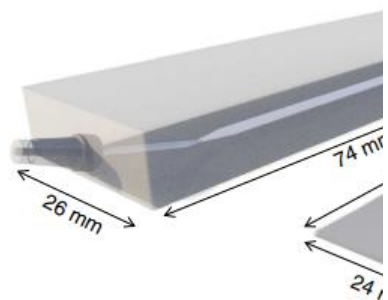
**Kim Jouffroy**



# Avancées majeures dans la compréhension des mécanismes moléculaires de l'hémostase primaire



Identification of platelet function defects by multi-parameter assessment of thrombus formation



**J. Cosemans**  
School for Cardiovascular Diseases  
Maastricht University