2016' VHRC 2nd Int. Symposium

Understanding of Vulnerable Blood with Blood Rheology

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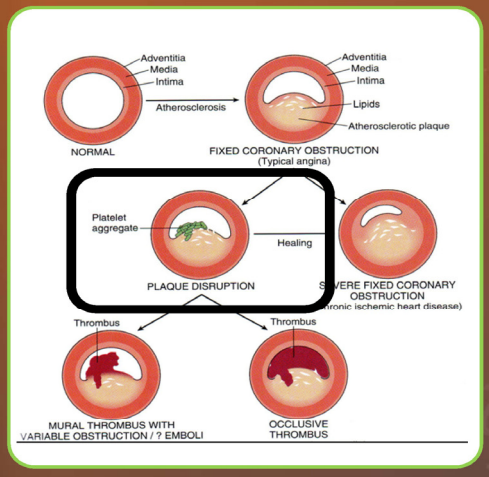
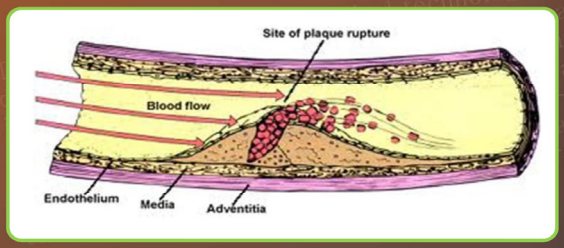
Vulnerable Plague

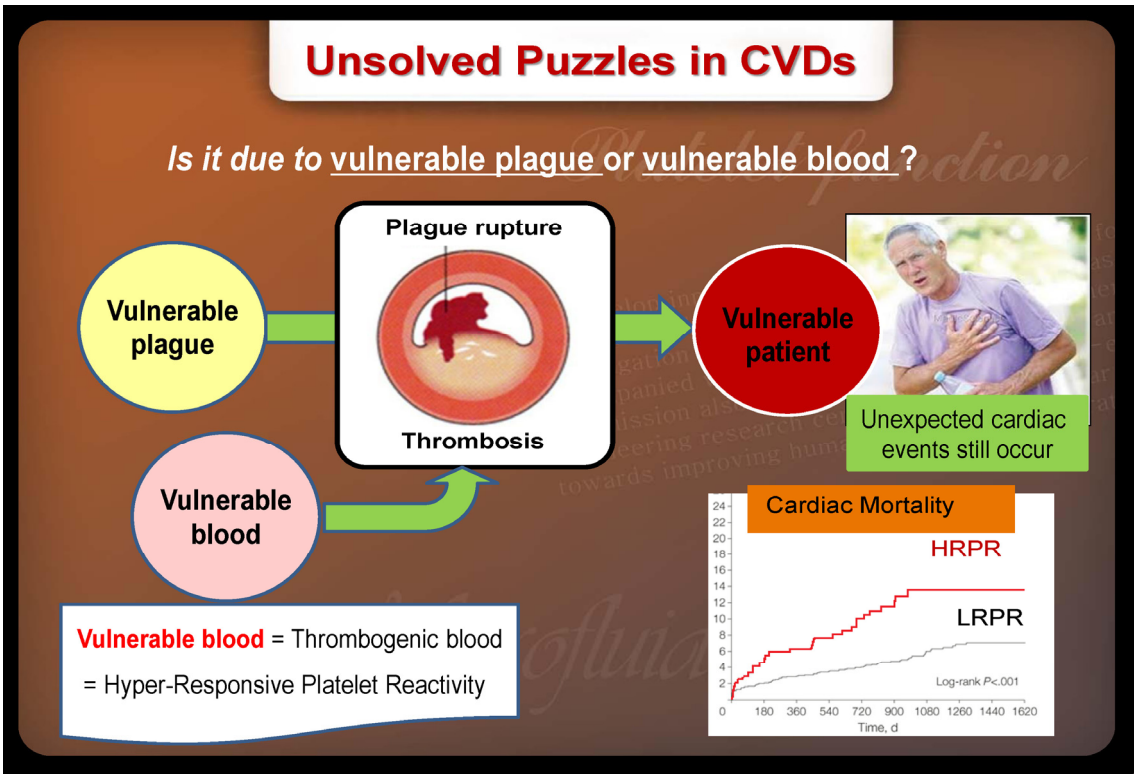
Atherosclerotic cardiovascular disease :
- a major cause of sudden deaths for several decades
- CAD is the most directly correlated with the results

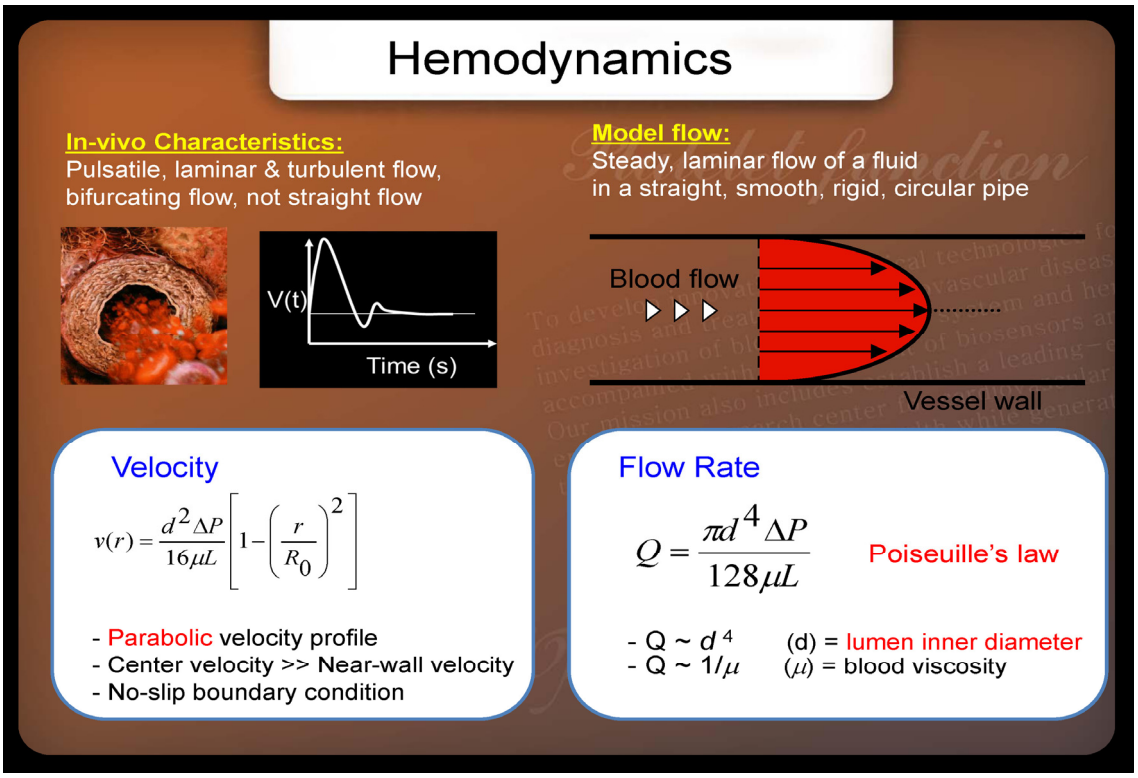




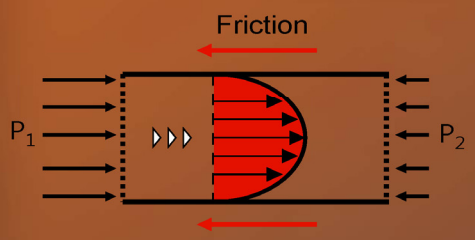
Unanswered Questions:

1. Plague is going to be ruptured soon or not ?
2. Plague rupture leads to occlusive thrombosis ?
3. How the clots would be lysed ?


Unsolved Puzzles in CVDs
Is it due to vulnerable plague or vulnerable blood ?
Vulnerable plague
Plague rupture
Thrombosis
Vulnerable blood
Vulnerable patient
Unexpected cardiac events still occur
Cardiac Mortality
HRPR
LRPR
Log-rank P<.001
Time, d
Vulnerable blood = Thrombogenic blood
= Hyper-Responsive Platelet Reactivity


Hemodynamics
In-vivo Characteristics:
Pulsatile, laminar & turbulent flow, bifurcating flow, not straight flow
V(t)
Time (s)
Model flow:
Steady, laminar flow of a fluid in a straight, smooth, rigid, circular pipe
Blood flow
Vessel wall
Velocity
$v(r) = \frac{d^2 \Delta P}{16 \mu L}\left[1 - \left(\frac{r}{R_0}\right)^2\right]$
- Parabolic velocity profile
- Center velocity >> Near-wall velocity
- No-slip boundary condition
Flow Rate
$Q = \frac{\pi d^4 \Delta P}{128 \mu L}$
Poiseuille's law
- Q ~ d^4 (d) = lumen inner diameter
- Q ~ $1/\mu$ (μ) = blood viscosity

Hemodynamics (2)



Pressure (P)

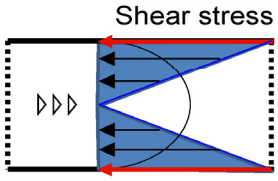
A flow energy to drive fluid to move through vessel

- unit: [Pa] or [mmHg]
- same as that of stress (in fact, pressure is a **normal stress**)

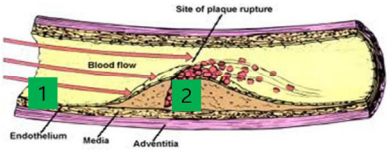
Shear Stress (τ)

- a frictional force on the wall in opposite direction of flow

$$\tau = \mu \frac{dv}{dr}$$



High Shear-induced *Thrombosis*



1. Mass Conservation

$Q = A_1V_1 = A_2V_2 = \text{const}$

-V_2 : increase with $1/d^2$

2. Bernoulli's' Law

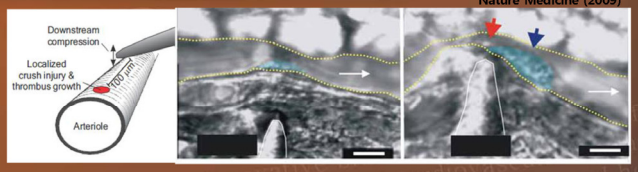
$$\frac{p_1}{\rho} + \frac{1}{2}V_1^2 = \frac{p_2}{\rho} + \frac{1}{2}V_2^2 = \text{Const}$$

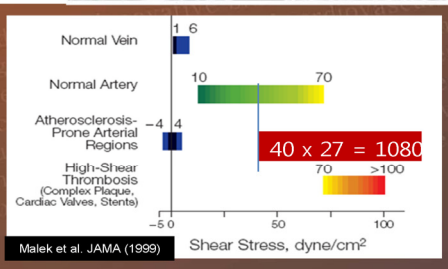
-P_2 : decreases (*spasm* may occur)

3. Shear stress ~ $(1/d)^3$

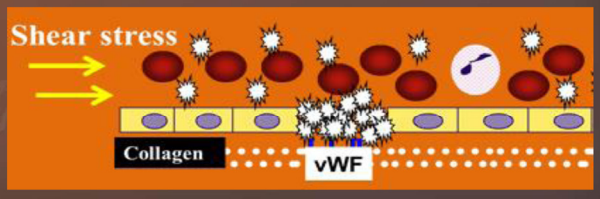
$$\tau = \frac{32\mu Q}{\pi d^3}$$

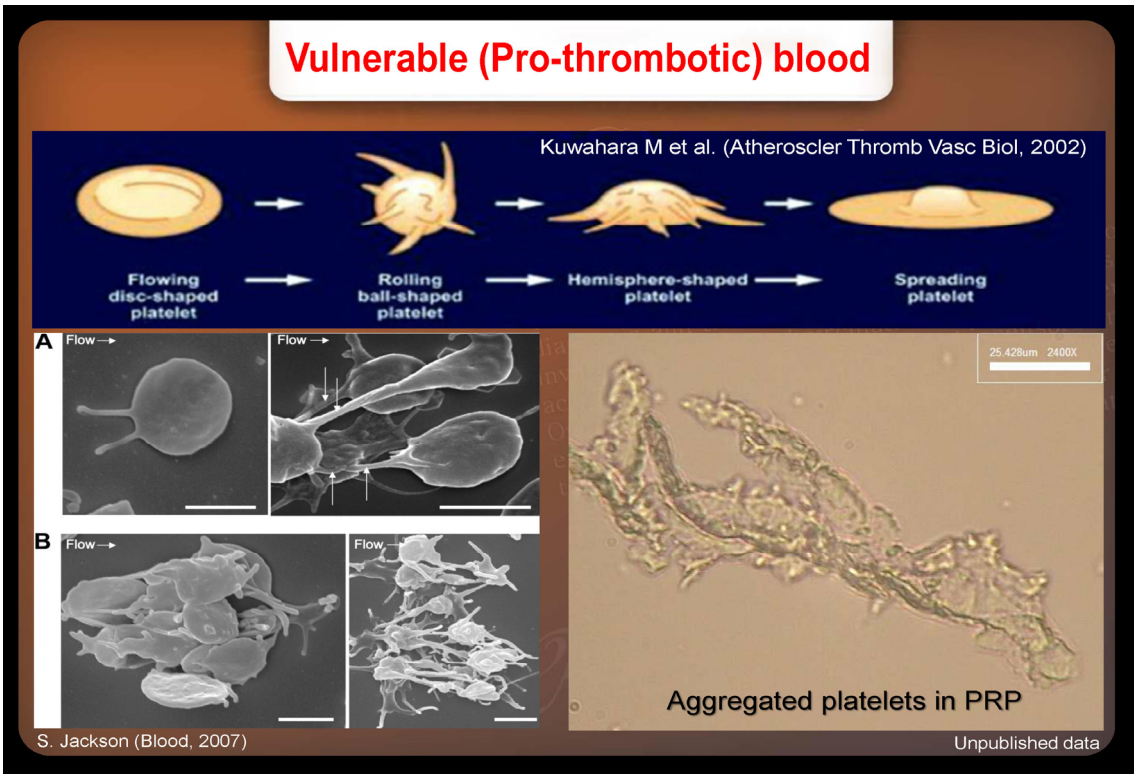
Nature Medicine (2009)

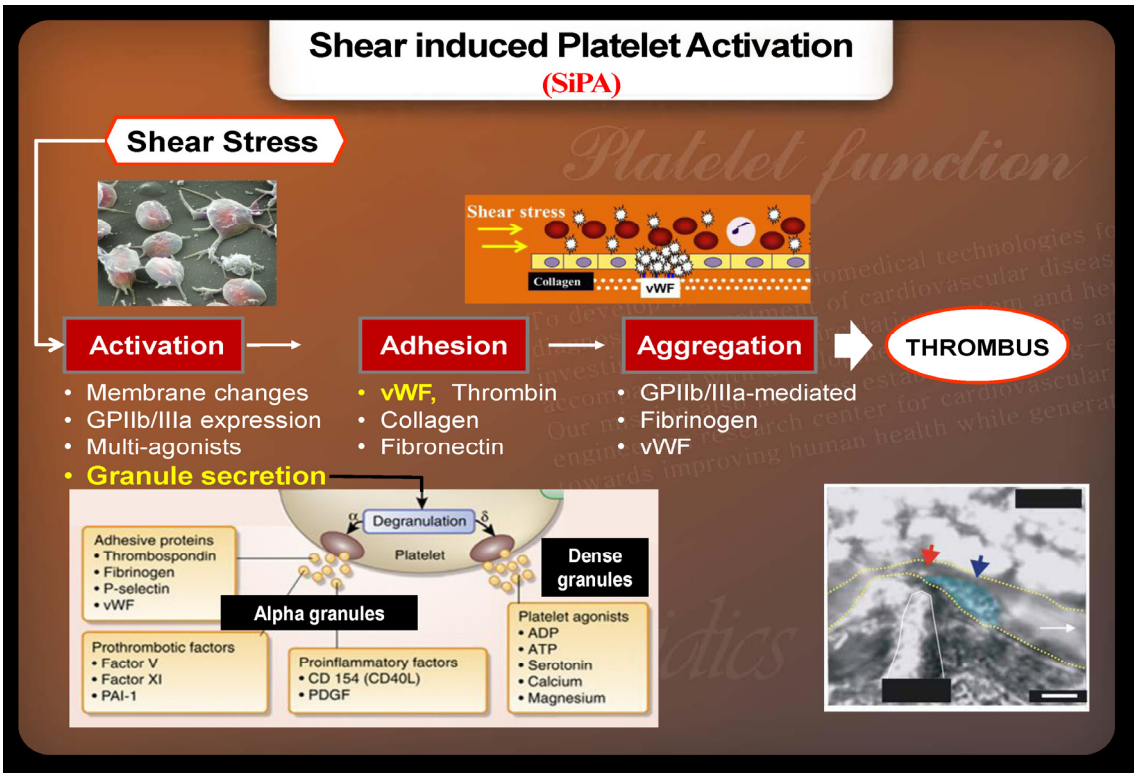


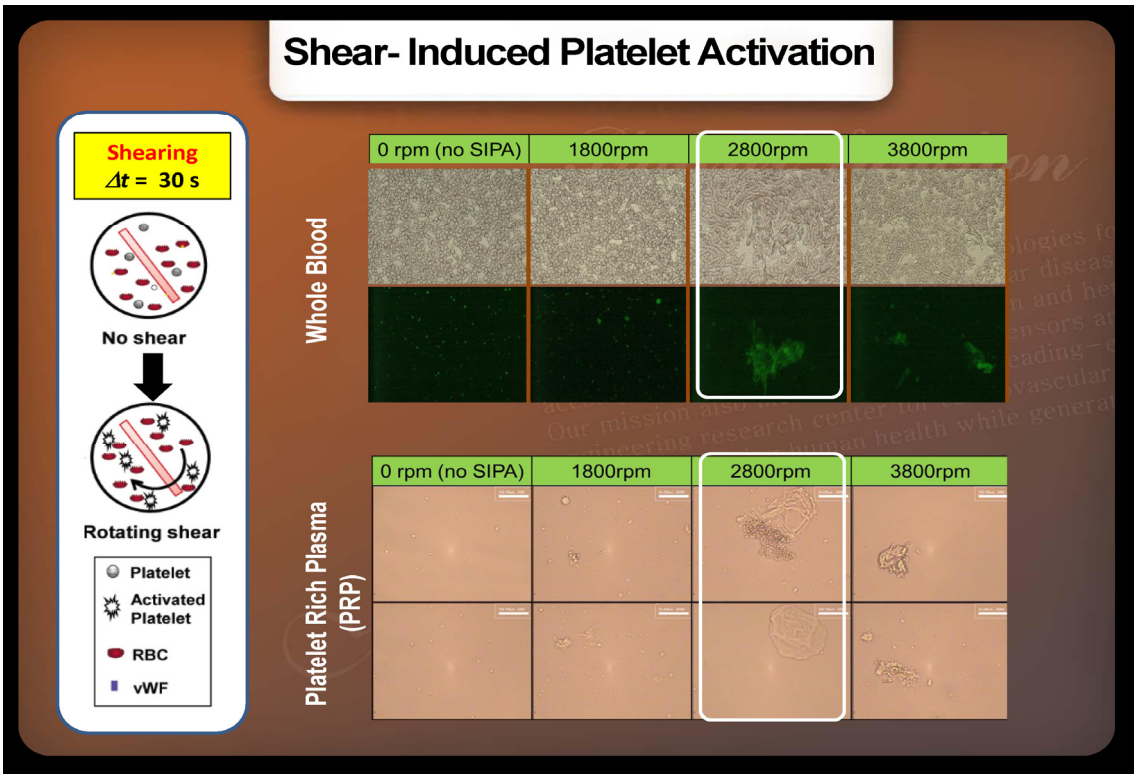


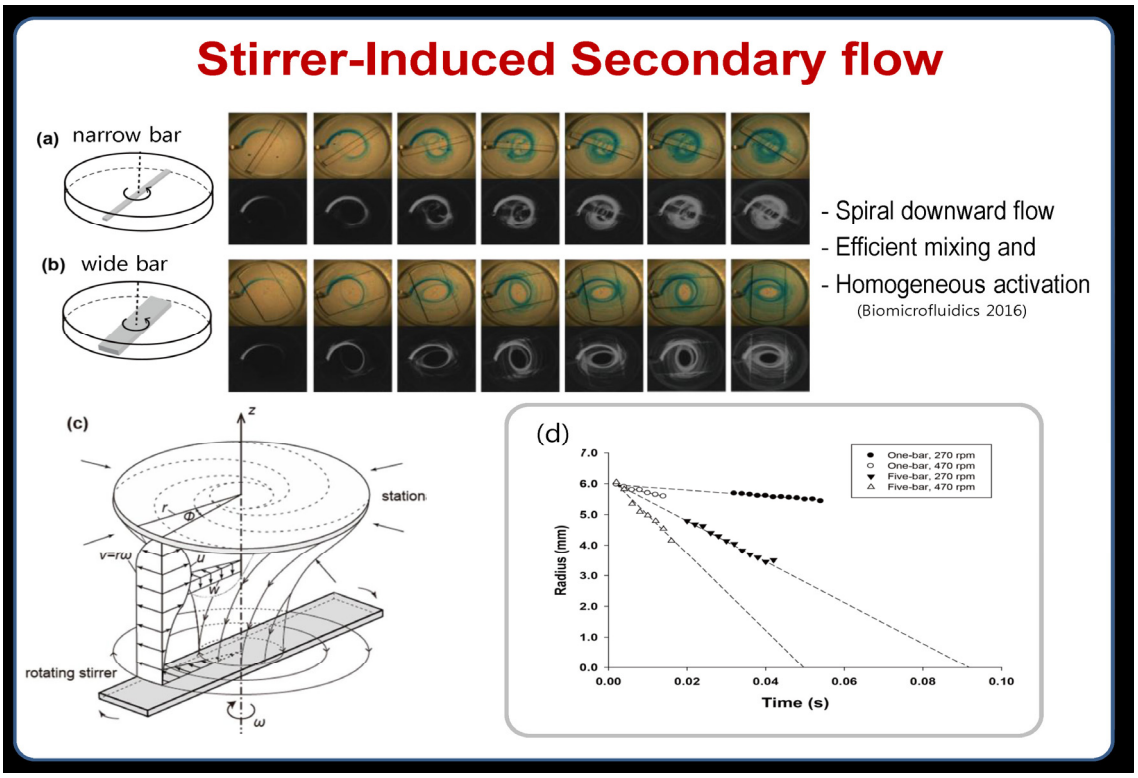
Malek et al. JAMA (1999)

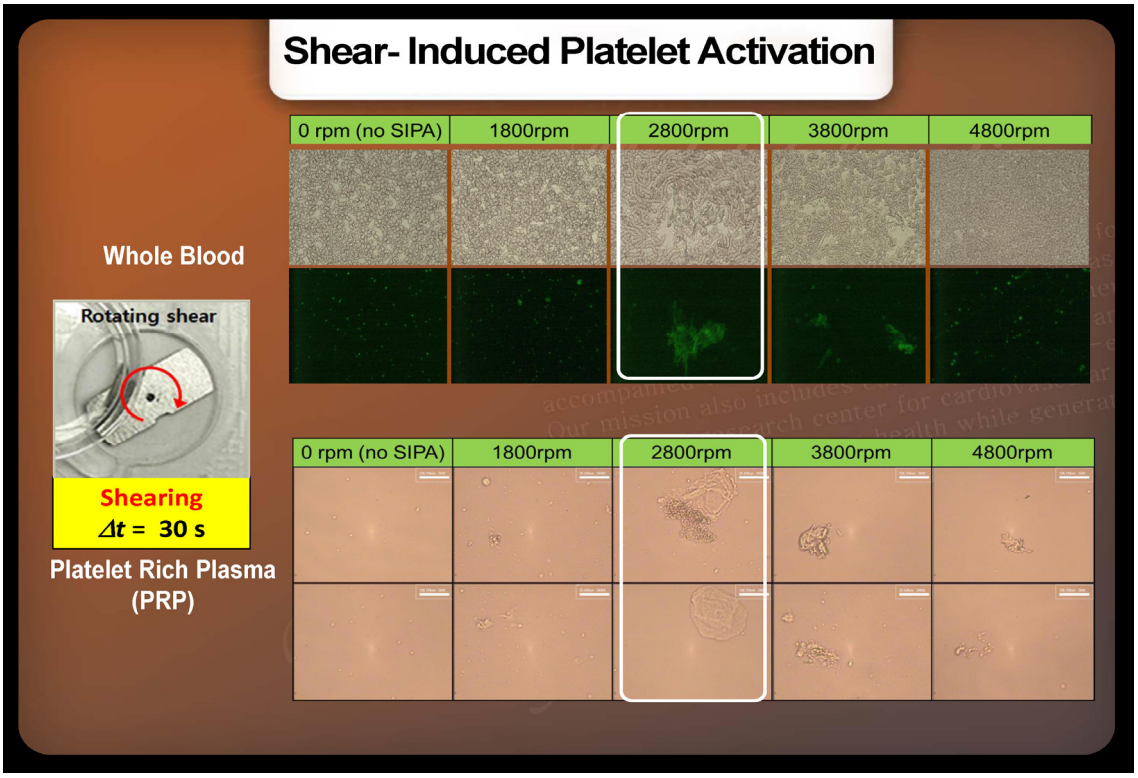


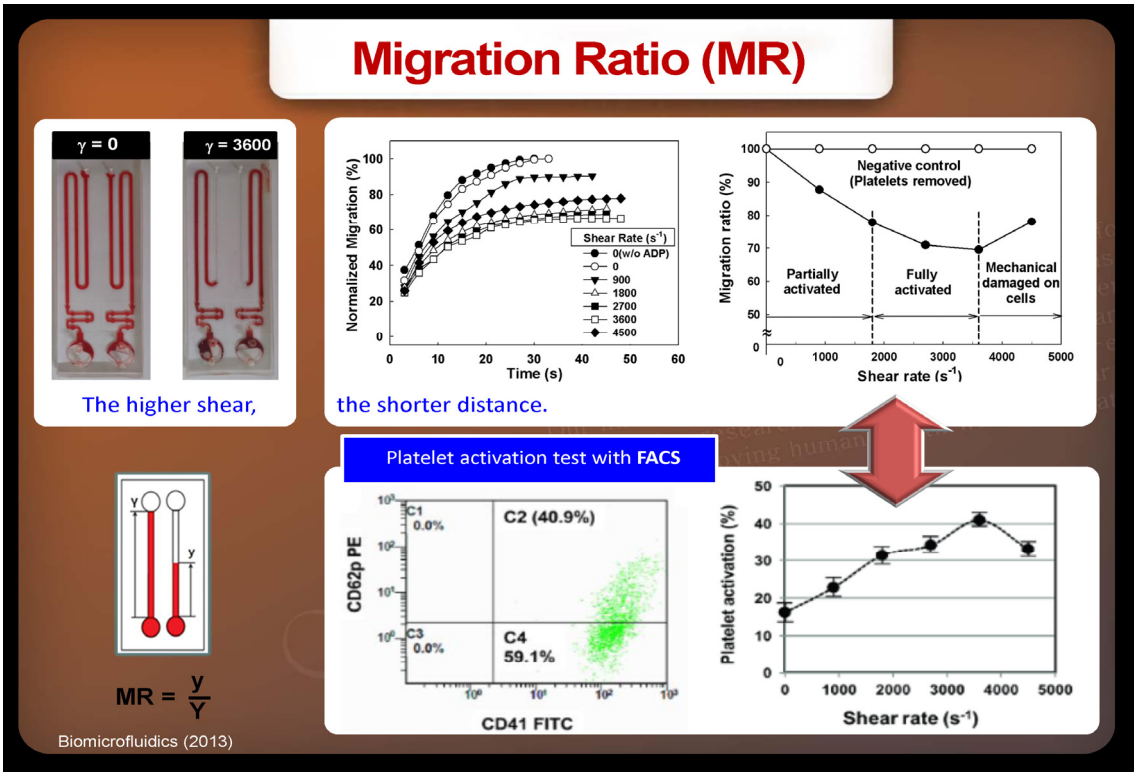

Vulnerable (Pro-thrombotic) blood
Kuwahara M et al. (Atheroscler Thromb Vasc Biol, 2002)
Flowing disc-shaped platelet
Rolling ball-shaped platelet
Hemisphere-shaped platelet
Spreading platelet
A
B
Flow
Aggregated platelets in PRP
S. Jackson (Blood, 2007)
Unpublished data

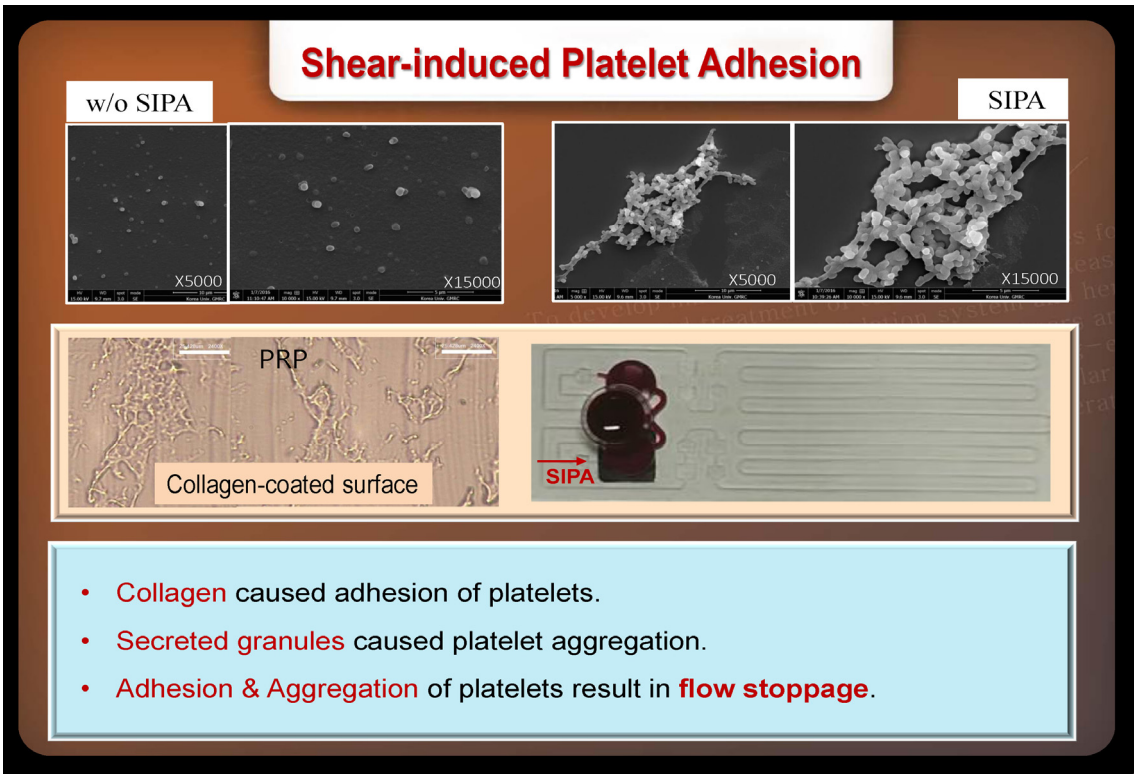

Shear induced Platelet Activation
(SiPA)
Shear Stress
Shear stress
Collagen
vWF
Activation
Adhesion
Aggregation
THROMBUS
• Membrane changes
• GPIIb/IIIa expression
• Multi-agonists
• Granule secretion
• vWF, Thrombin
• Collagen
• Fibronectin
• GPIIb/IIIa-mediated
• Fibrinogen
• vWF
Degranulation
Platelet
Adhesive proteins
• Thrombospondin
• Fibrinogen
• P-selectin
• vWF
Alpha granules
Dense granules
Prothrombotic factors
• Factor V
• Factor XI
• PAI-1
Proinflammatory factors
• CD 154 (CD40L)
• PDGF
Platelet agonists
• ADP
• ATP
• Serotonin
• Calcium
• Magnesium

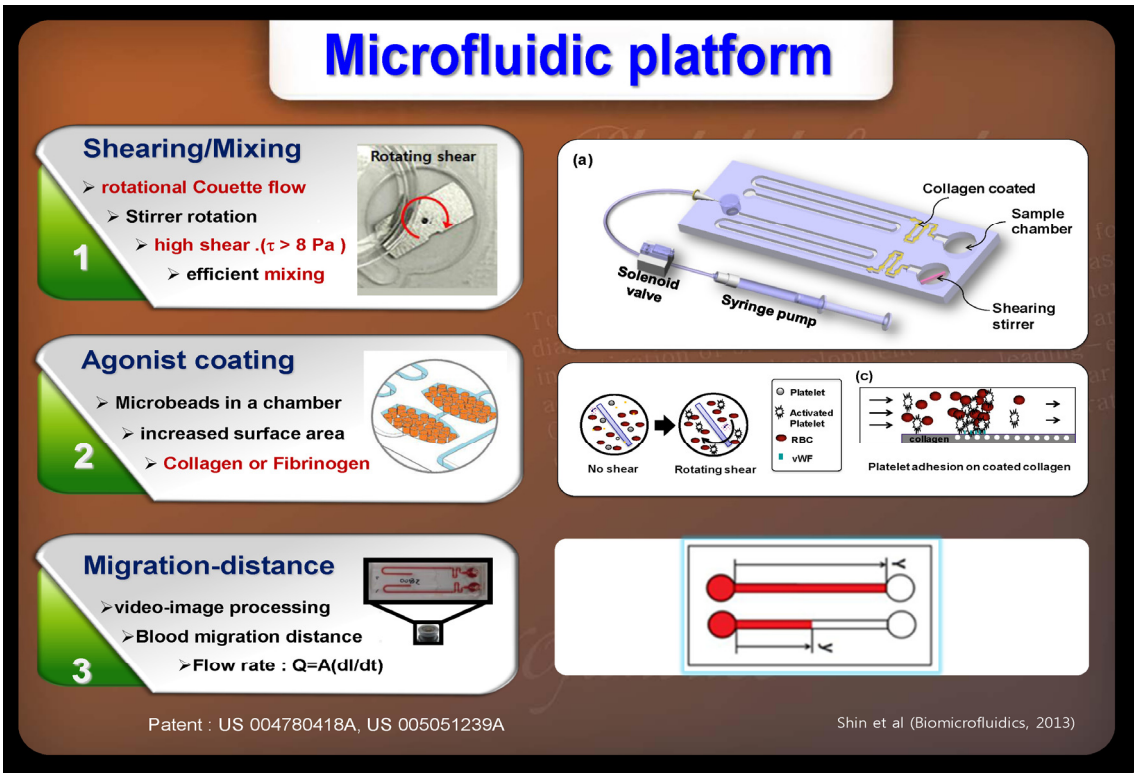

Shear- Induced Platelet Activation
Shearing
Δt = 30 s
No shear
Rotating shear
Platelet
Activated Platelet
RBC
vWF
Whole Blood
Platelet Rich Plasma (PRP)
0 rpm (no SIPA)
1800rpm
2800rpm
3800rpm

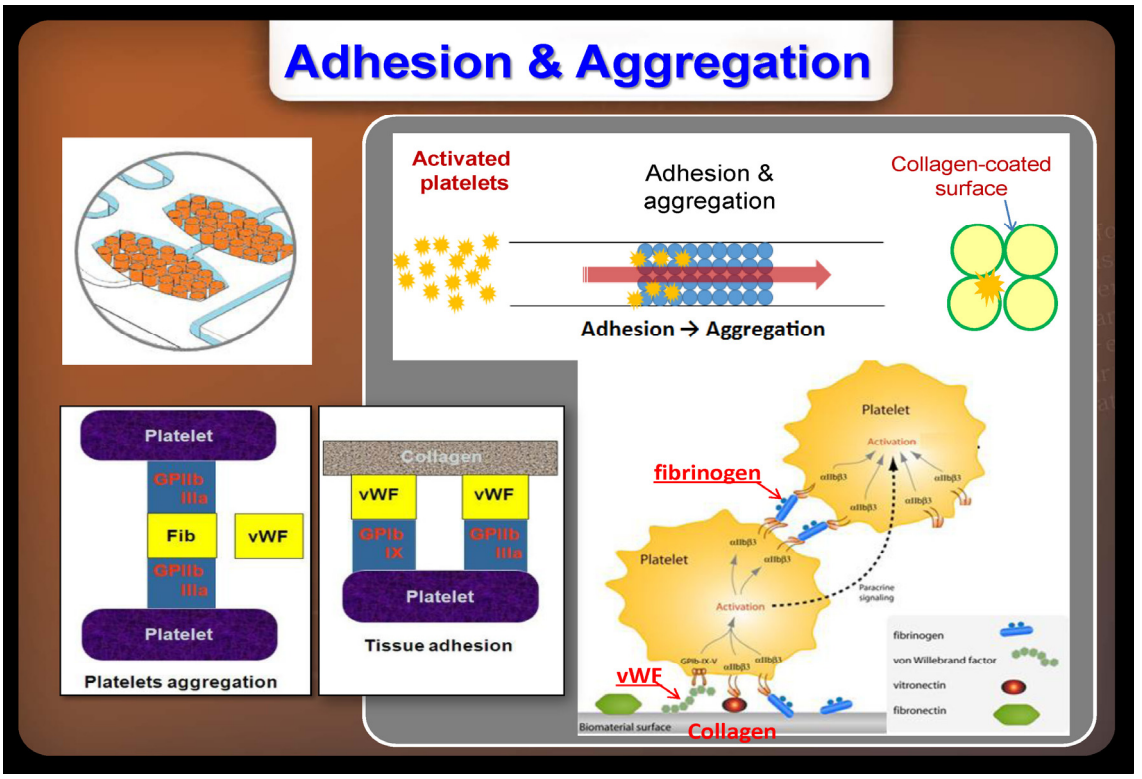

Stirrer-Induced Secondary flow
(a) narrow bar
(b) wide bar
- Spiral downward flow
- Efficient mixing and
- Homogeneous activation
(Biomicrofluidics 2016)
(c)
station
rotating stirrer
(d)
One-bar, 270 rpm
One-bar, 470 rpm
Five-bar, 270 rpm
Five-bar, 470 rpm
Radius (mm)
Time (s)

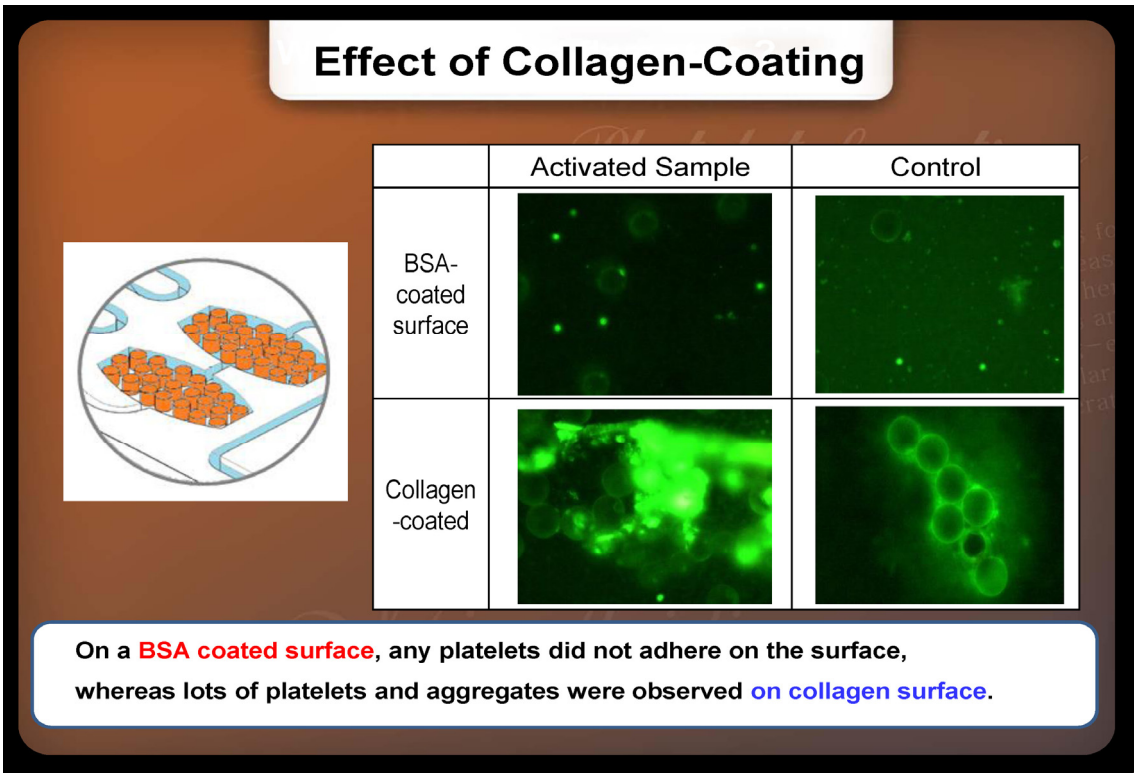

Shear- Induced Platelet Activation
0 rpm (no SIPA)
1800rpm
2800rpm
3800rpm
4800rpm
Whole Blood
Rotating shear
Shearing
Δt = 30 s
Platelet Rich Plasma
(PRP)
0 rpm (no SIPA)
1800rpm
2800rpm
3800rpm
4800rpm

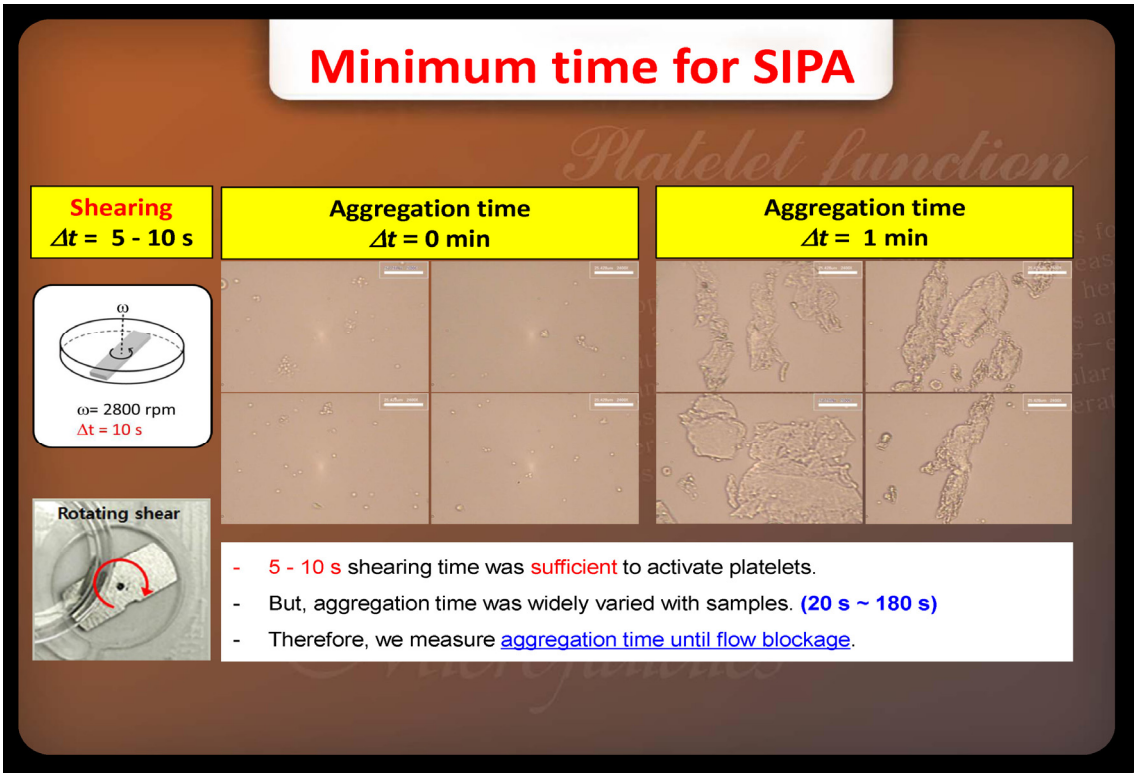

Migration Ratio (MR)
γ = 0
γ = 3600
The higher shear,
Normalized Migration (%)
Time (s)
Shear Rate (s-1)
0(w/o ADP)
0
900
1800
2700
3600
4500
the shorter distance.
Migration ratio (%)
Negative control
(Platelets removed)
Partially activated
Fully activated
Mechanical damaged on cells
Shear rate (s-1)
Platelet activation test with FACS
CD62p PE
CD41 FITC
C1 0.0%
C2 (40.9%)
C3 0.0%
C4 59.1%
Platelet activation (%)
Shear rate (s-1)
MR = y/Y
Biomicrofluidics (2013)

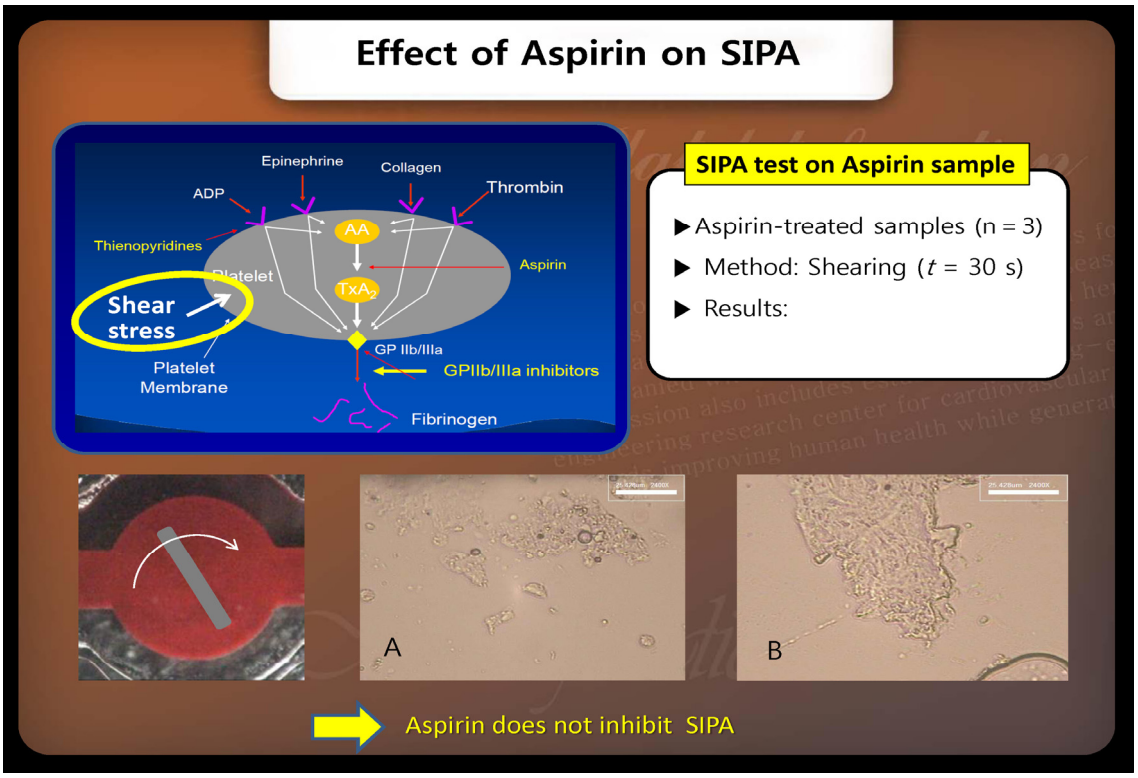

Shear-induced Platelet Adhesion
w/o SIPA
SIPA
X5000
X15000
X5000
X15000
PRP
Collagen-coated surface
SIPA
• Collagen caused adhesion of platelets.
• Secreted granules caused platelet aggregation.
• Adhesion & Aggregation of platelets result in flow stoppage.

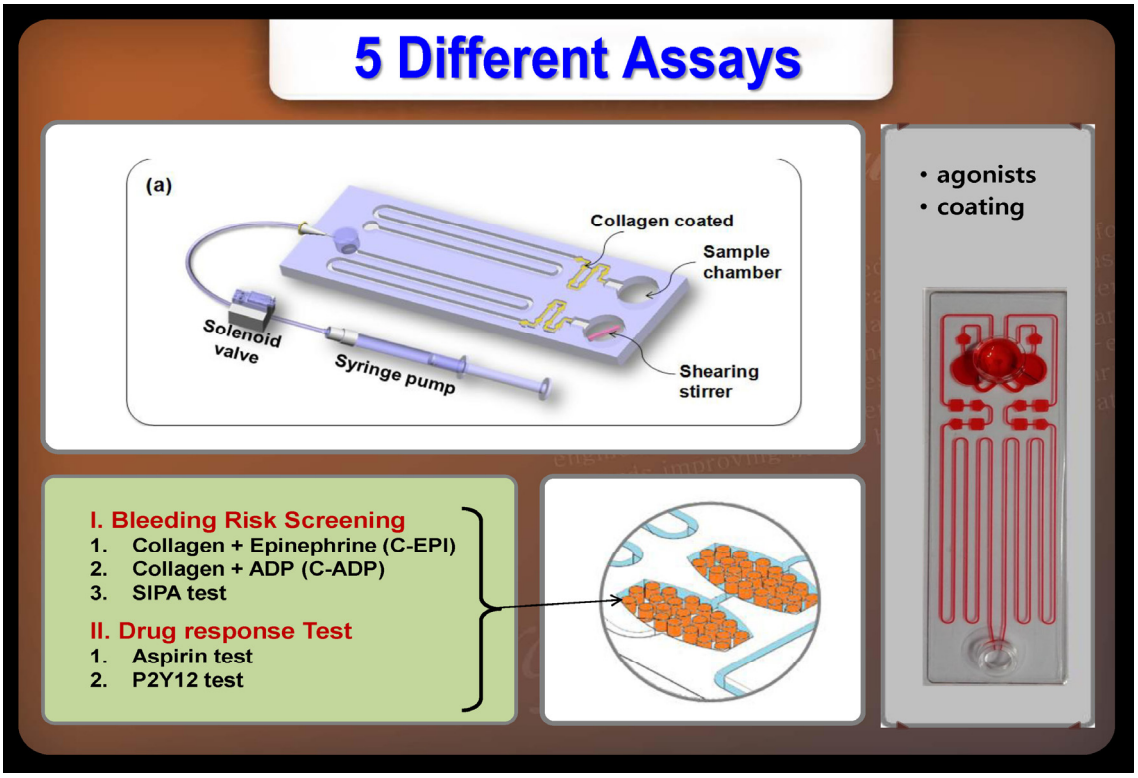

Microfluidic platform
Shearing/Mixing
➢ rotational Couette flow
➢ Stirrer rotation
➢ high shear .(τ > 8 Pa)
➢ efficient mixing
1
Rotating shear
Agonist coating
➢ Microbeads in a chamber
➢ increased surface area
➢ Collagen or Fibrinogen
2
Migration-distance
➢video-image processing
➢Blood migration distance
➢Flow rate : Q=A(dl/dt)
3
(a)
Collagen coated
Sample chamber
Solenoid valve
Syringe pump
Shearing stirrer
(c)
No shear
Rotating shear
Platelet
Activated Platelet
RBC
vWF
collagen
Platelet adhesion on coated collagen
Patent : US 004780418A, US 005051239A
Shin et al (Biomicrofluidics, 2013)

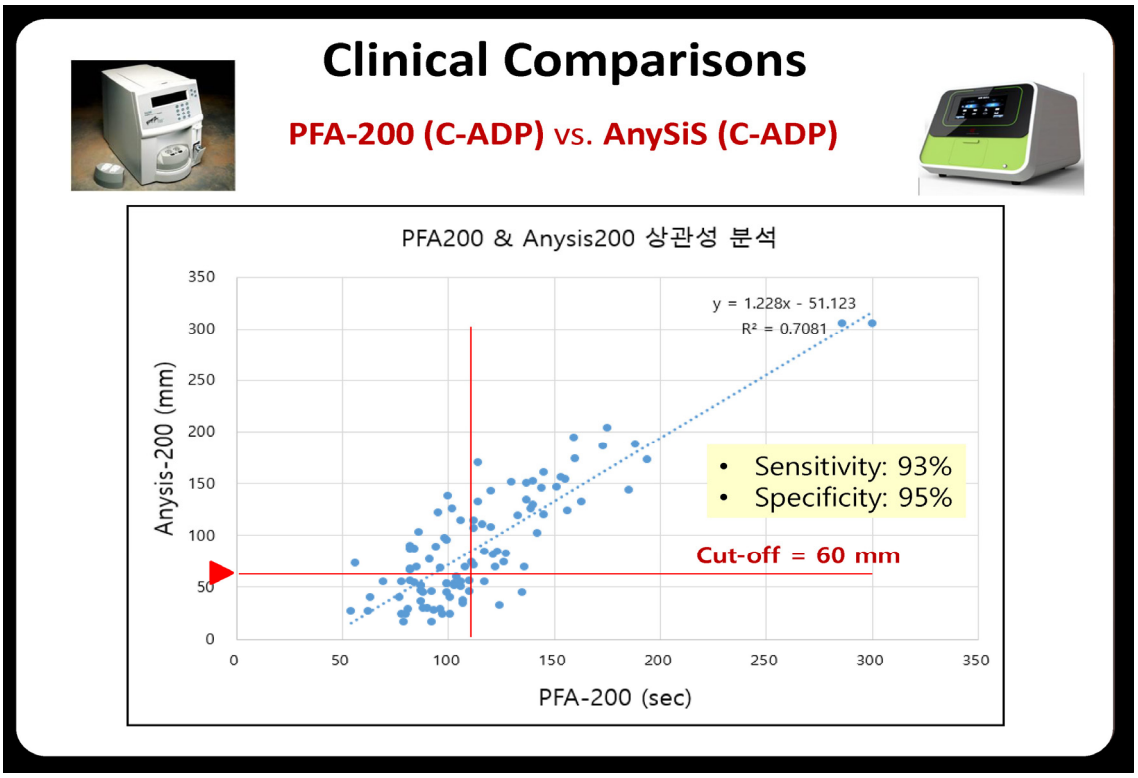

Adhesion & Aggregation
Activated platelets
Adhesion & aggregation
Collagen-coated surface
Adhesion → Aggregation
Platelet
GPIIb IIIa
Fib
vWF
GPIIb IIIa
Platelet
Platelets aggregation
Collagen
vWF
vWF
GPIb IX
GPIIb IIIa
Platelet
Tissue adhesion
Platelet
Activation
fibrinogen
Platelet
Activation
Paracrine signaling
vWF
Collagen
Biomaterial surface
fibrinogen
von Willebrand factor
vitronectin
fibronectin

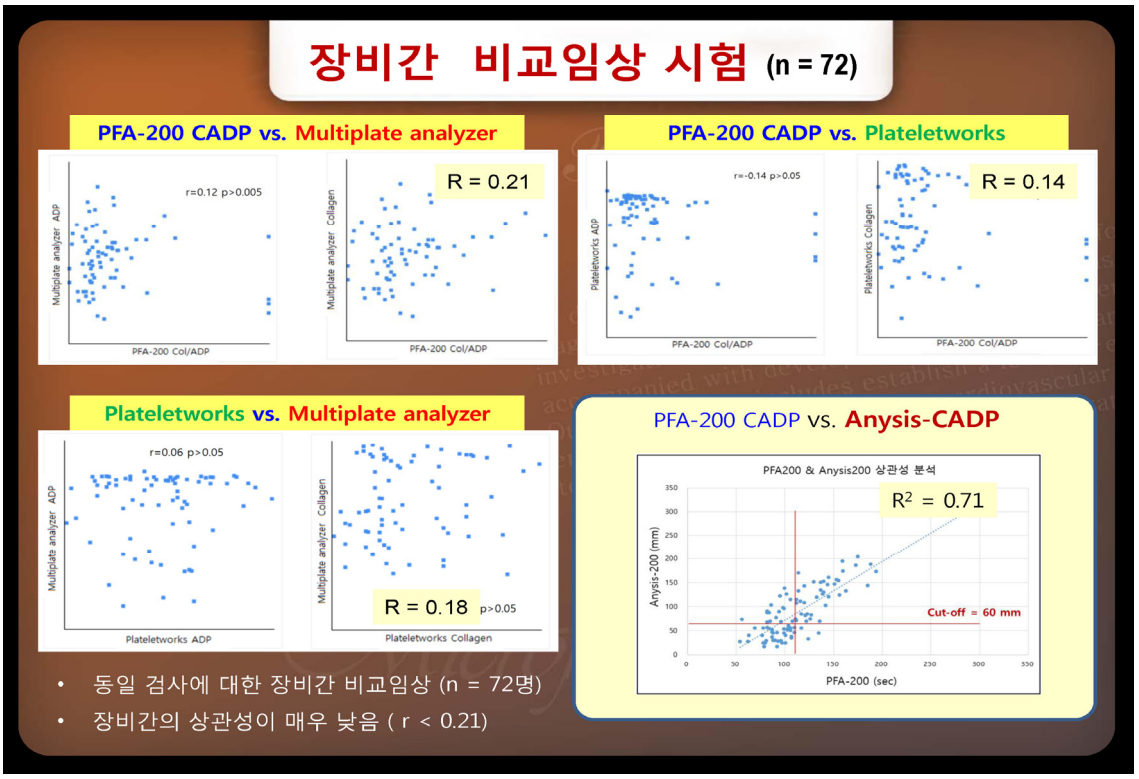

Effect of Collagen-Coating
Activated Sample
Control
BSA-coated surface
Collagen -coated
On a BSA coated surface, any platelets did not adhere on the surface,
whereas lots of platelets and aggregates were observed on collagen surface.


Minimum time for SIPA
Shearing
Δt = 5 - 10 s
Aggregation time
Δt = 0 min
Aggregation time
Δt = 1 min
ω
ω= 2800 rpm
Δt = 10 s
Rotating shear
- 5 - 10 s shearing time was sufficient to activate platelets.
- But, aggregation time was widely varied with samples. (20 s ~ 180 s)
- Therefore, we measure aggregation time until flow blockage.


Effect of Aspirin on SIPA
Epinephrine
Collagen
ADP
Thrombin
Thienopyridines
AA
Aspirin
Platelet
TxA2
Shear stress
GP IIb/IIIa
GPIIb/IIIa inhibitors
Platelet Membrane
Fibrinogen
SIPA test on Aspirin sample
▶Aspirin-treated samples (n = 3)
▶ Method: Shearing (t = 30 s)
▶ Results:
A
B
Aspirin does not inhibit SIPA


5 Different Assays
(a)
Collagen coated
Sample chamber
Solenoid valve
Syringe pump
Shearing stirrer
• agonists
• coating
I. Bleeding Risk Screening
1. Collagen + Epinephrine (C-EPI)
2. Collagen + ADP (C-ADP)
3. SIPA test
II. Drug response Test
1. Aspirin test
2. P2Y12 test

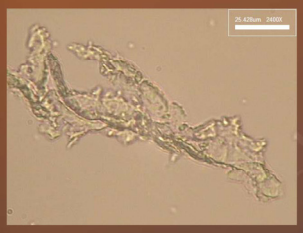

Clinical Comparisons
PFA-200 (C-ADP) vs. AnySiS (C-ADP)
PFA200 & Anysis200 상관성 분석
y = 1.228x - 51.123
R² = 0.7081
• Sensitivity: 93%
• Specificity: 95%
Cut-off = 60 mm
Anysis-200 (mm)
PFA-200 (sec)
0
50
100
150
200
250
300
350

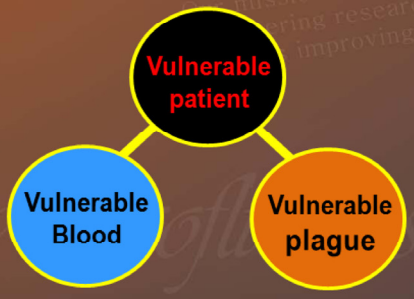

장비간 비교임상 시험 (n = 72)
PFA-200 CADP vs. Multiplate analyzer
r=0.12 p>0.005
Multiplate analyzer ADP
PFA-200 Col/ADP
R = 0.21
Multiplate analyzer Collagen
PFA-200 Col/ADP
PFA-200 CADP vs. Plateletworks
r=-0.14 p>0.05
Plateletworks ADP
PFA-200 Col/ADP
R = 0.14
Plateletworks Collagen
PFA-200 Col/ADP
Plateletworks vs. Multiplate analyzer
r=0.06 p>0.05
Multiplate analyzer ADP
Plateletworks ADP
R = 0.18 p>0.05
Multiplate analyzer Collagen
Plateletworks Collagen
PFA-200 CADP vs. Anysis-CADP
PFA200 & Anysis200 상관성 분석
R2 = 0.71
Anysis-200 (mm)
Cut-off = 60 mm
PFA-200 (sec)
• 동일 검사에 대한 장비간 비교임상 (n = 72명)
• 장비간의 상관성이 매우 낮음 (r < 0.21)

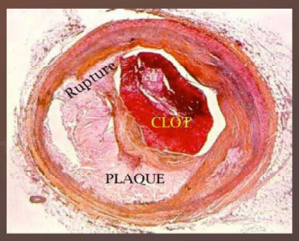

Anysis-ONE
I. 혈소판 검사 기기 (1등급)
Anysis-ONE
II. 혈소판 복합기능 검사 칩 (3등급)
RheoScan-PA1: COL/ADP
II. 항혈소판 약물반응 검사 칩 (3등급)
RheoScan-PA2: Aspirin test
RheoScan
Reagent A
Reagent C
Reagent E

Conclusions

- **Shear stress at stenosed section** is significantly increased, proportional to $(1/d)^3$.
- Shear stress is a strong agonistic factor to activate platelets.
- SIPA requires relatively short time (t < 5 s) but sufficient shear stress (t > 8 Pa)
- Aspirin cannot inhibit SIPA and its consequences
- Clotted blood could be naturally lysed with tPA.
- Therefore, it may be required to test thrombolysis as well as thrombosis.







Acknowledgements

Collaborators (I)

- Kim, Yeul-Hong (M.D., Korea U) Clinical validation
- Kang, Jae-woo (Korea U.) : Big Data analysis
- Lee, Ho-Young (SNU): Drug Resistance
- Lee, Hyuk-jin (Ewha Women U.): Drug Delivery
- Lee, Jae-Seung (Korea Univ.): Nano plasmonics
- Na, Sung-soo (Korea U.) QCM sensor
- Han, Chang-Soo (Korea U.) Nano-patterning
- Park, KyungWha (Korea U) Clinical validation
- Bong, Ki-Wan (Korea U) : Flow Lithography
- Lee, WonJong (Incheon U) : Exosome
- Kim, SungJin (Konkuk U) : Microfluidics

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Collaborators (II)

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