

Platelet Function Measurements

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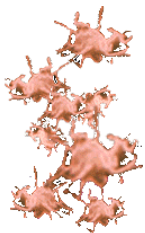
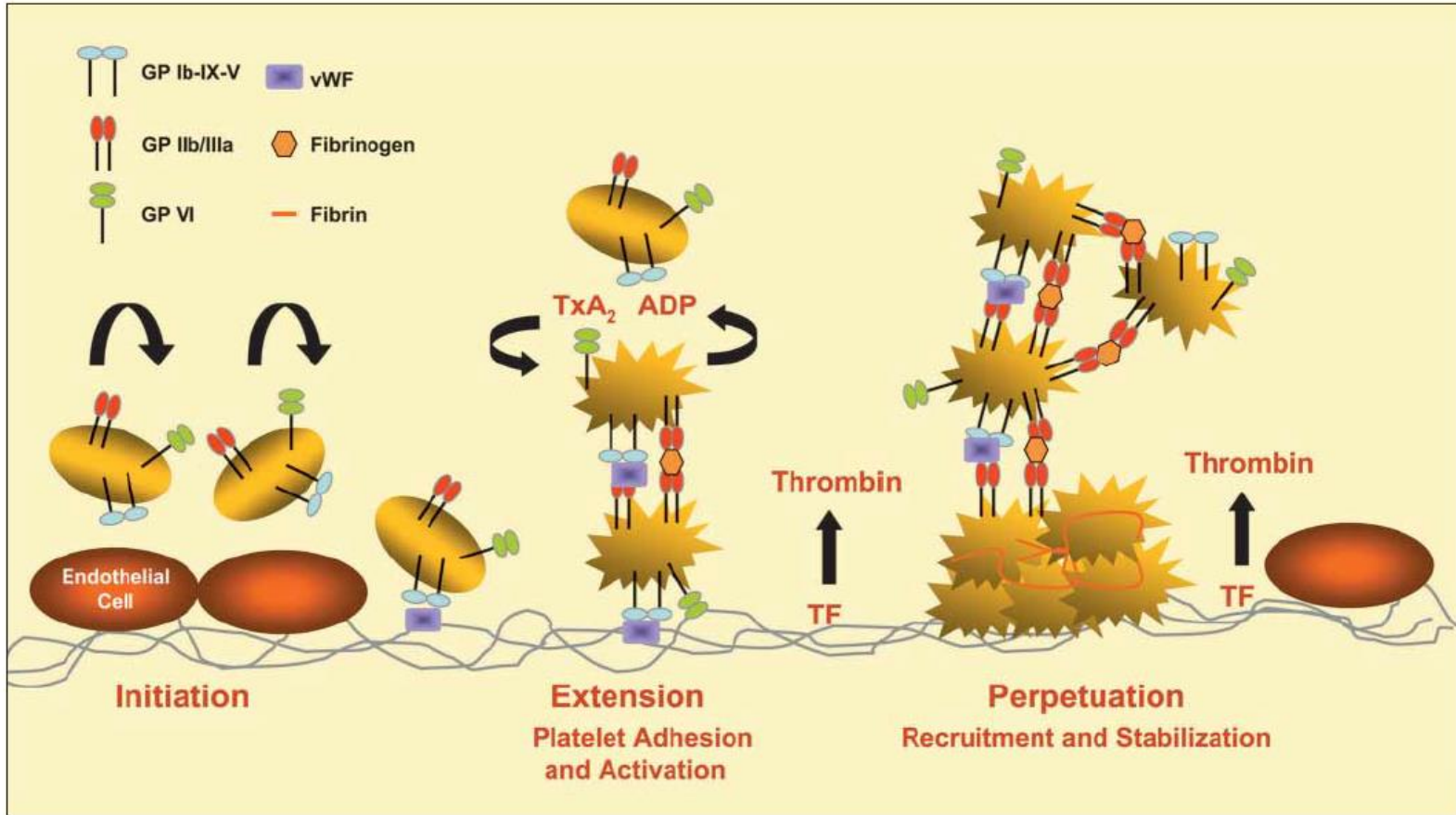


OUTLINE

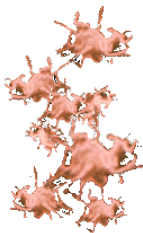
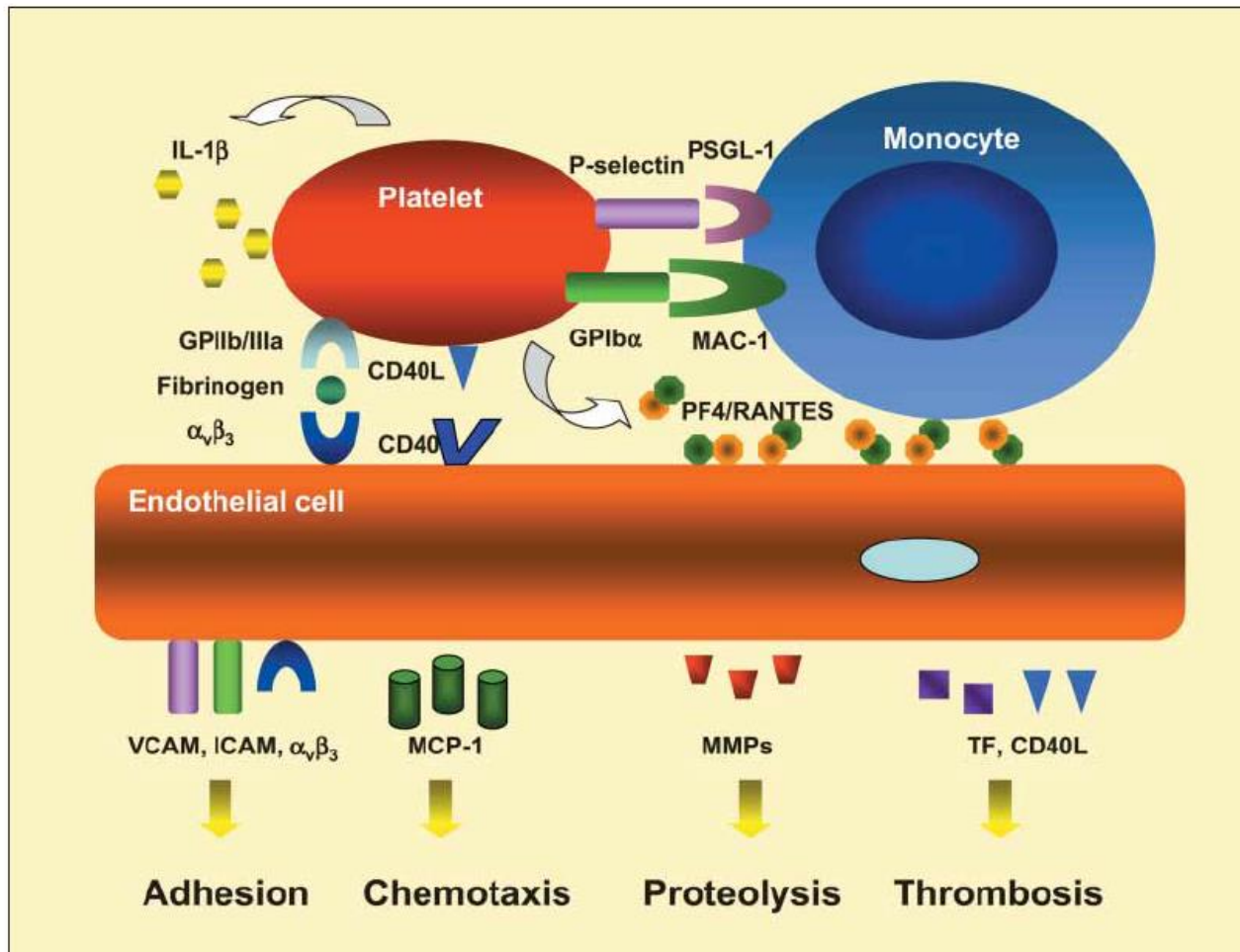
- Role of Platelets in Hemostasis
- Platelet Function Tests
- Future Directions



Platelet Plug Formation

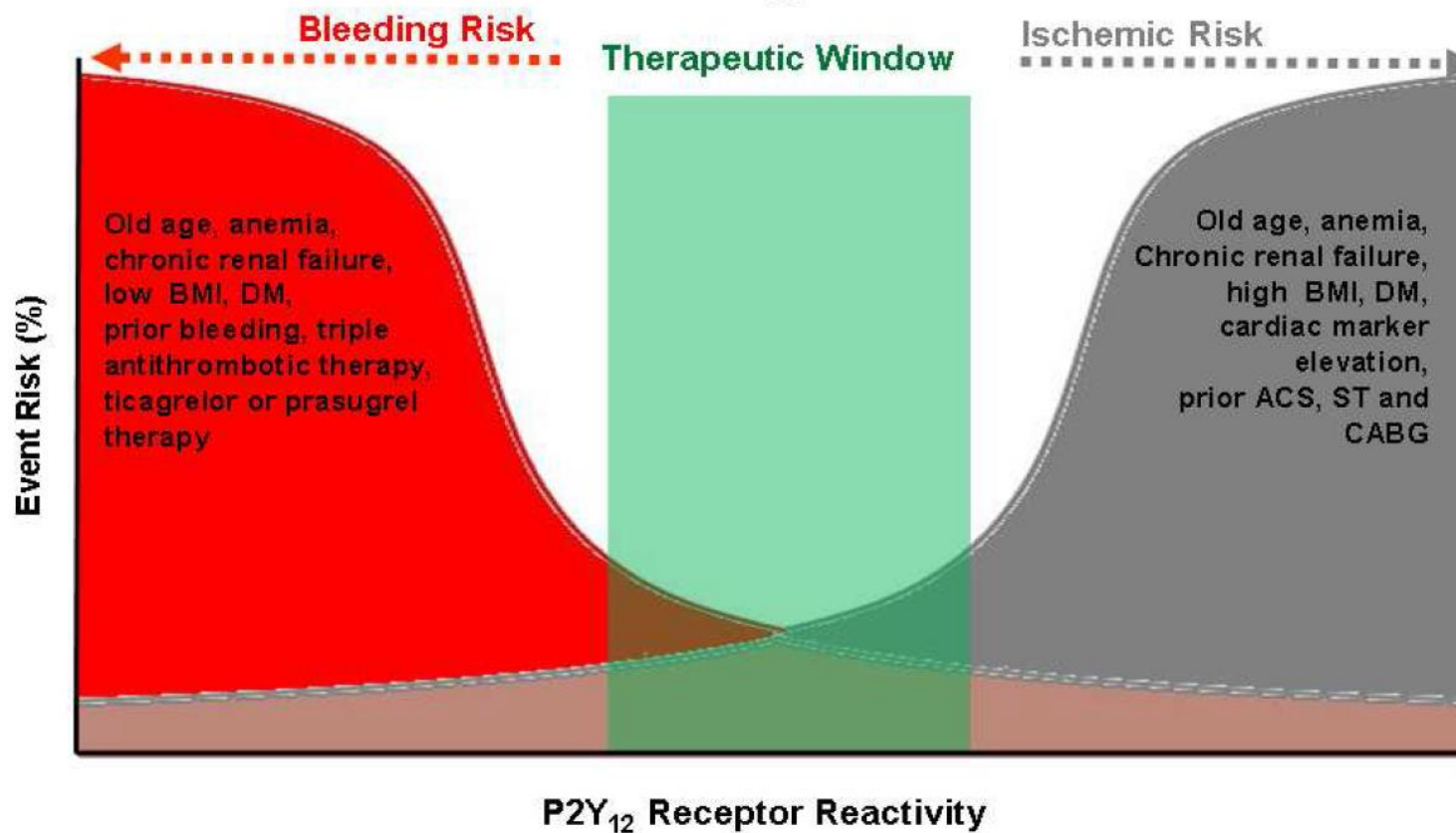


Endothelial Cells & Monocytes



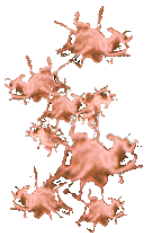
Therapeutic Window Concept

<85 VerifyNow-PRU >208
<16% VASP-PRI >50%
<19 MEA-AU*min >46
<31 TEG-MA_{ADP} (mm) >47



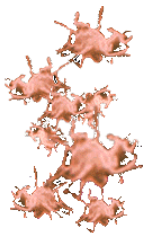
Platelet Function Tests

- Traditionally used for aid in the diagnosis and management of patients with bleeding problems.
- Given the role in atherothrombosis, several instruments are now available as point-of-care (POC) assays.
- Some can be used to monitor antiplatelet therapy and assess risk of bleeding and thrombosis, although still needs guidelines.



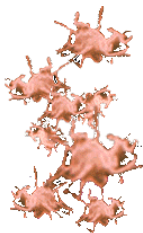
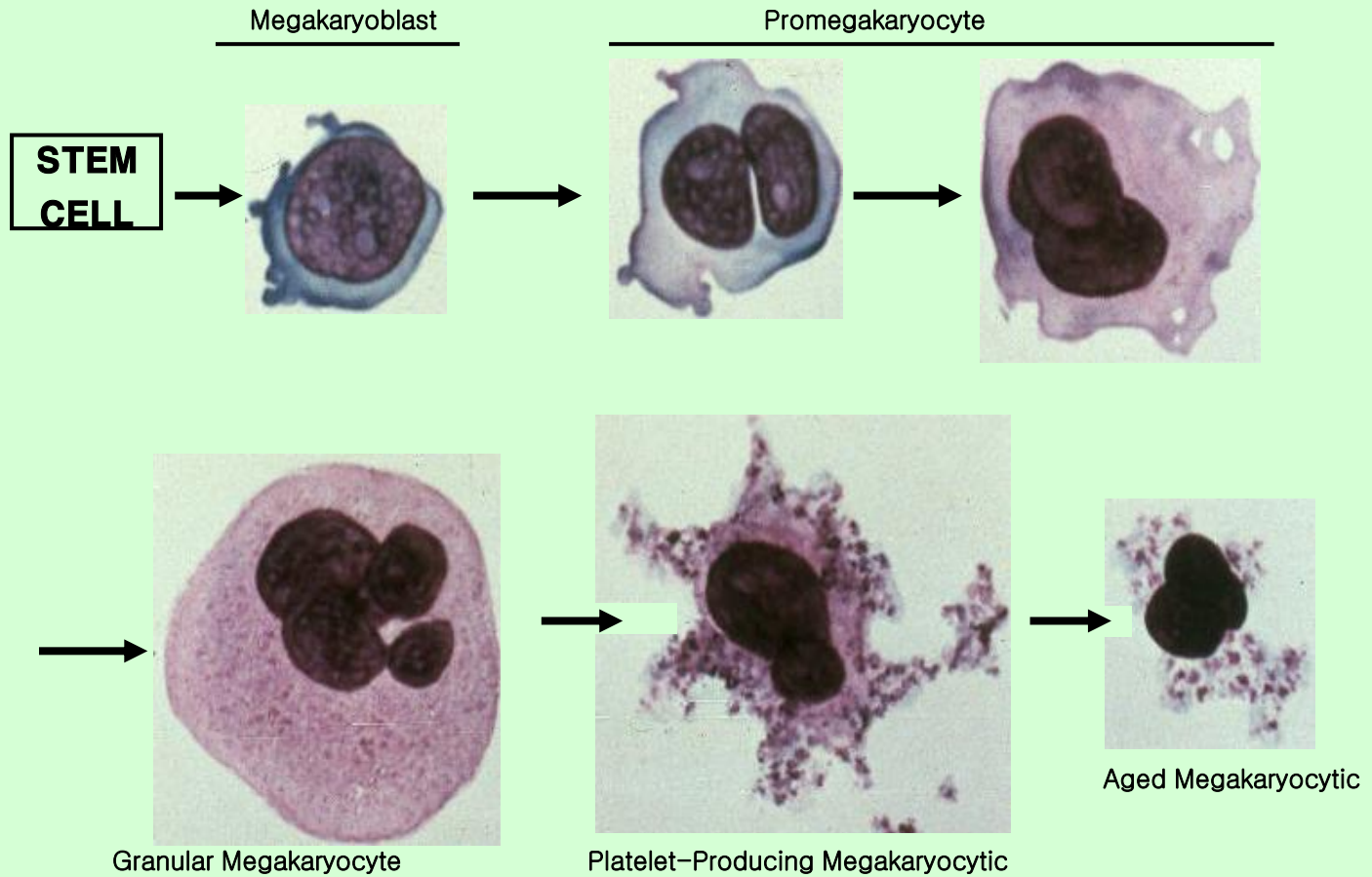
Platelet Counting & Morphology

- Classically evaluated by hematology analyzers
- Impedance, optical or immunological methods
- EDTA-anticoagulated blood
- Blood smears (May-Grünwald-Giemsa)
- Electron microscopy

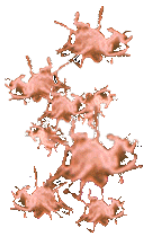
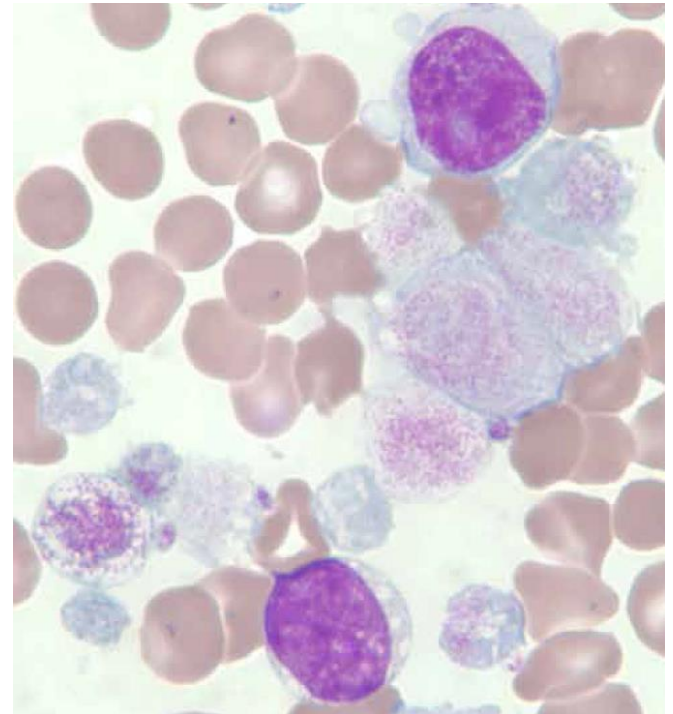
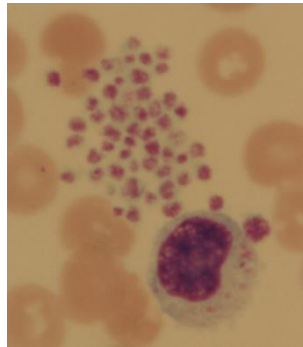
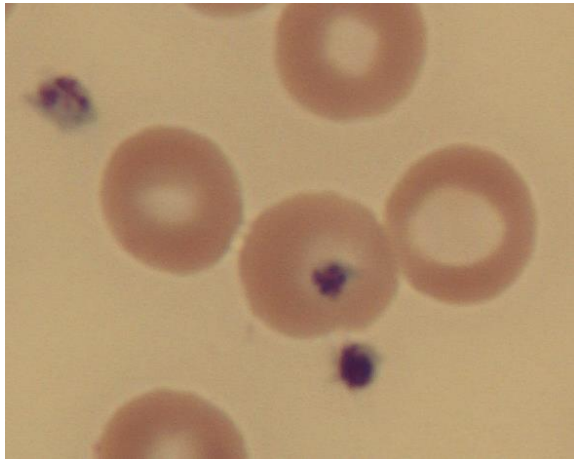
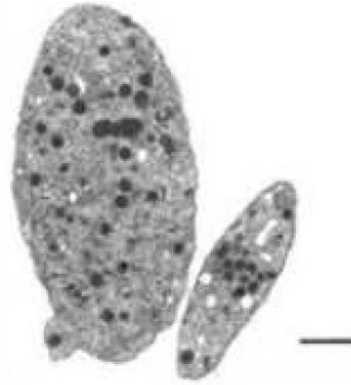
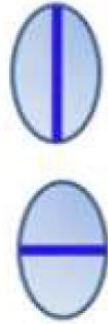
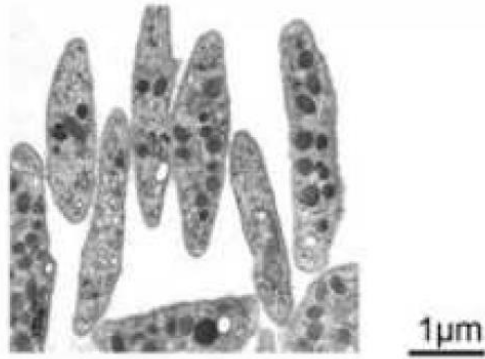


Platelet Counting & Morphology

Maturation of the Megakaryocytic

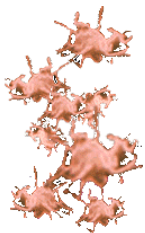


Platelet Counting & Morphology



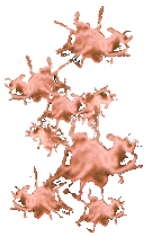
Platelet Aggregometry

- Still gold standard of platelet function testing
- Platelet aggregation is measured by analysis of the transmittance of light through a sample PRP
- Recently multichannel capability, computer operation, measuring secretion of nucleotides
- Nonphysiologic, influenced by many variables, requiring large blood volumes

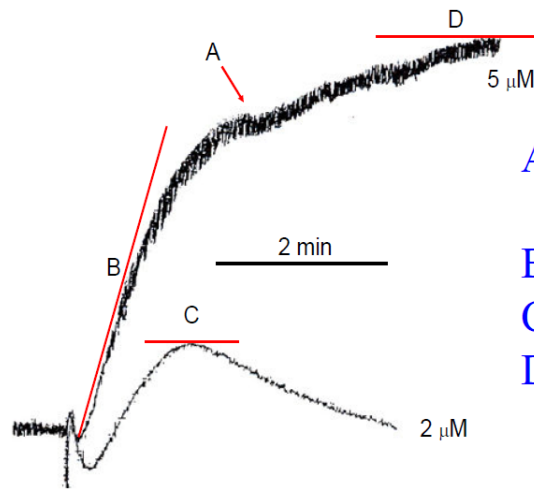


Platelet Aggregometry

- Choice of agonists (SSC/ISTH, 2013)
 - ADP: 2 μ M
 - Epinephrine: 5 μ M
 - Collagen: 2 μ g/mL
 - Thrombin receptor activating peptide (PAS1-AP): 10 μ M
 - Thromboxane A₂ mimetic (U46619): 1 μ M
 - Arachidonic acid: 1 mM
 - Ristocetin: 1.2 mg/mL
- Higher concentration should be used if abnormal results with the indicated concentration of each agonist.



Platelet Aggregometry



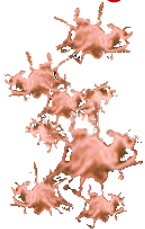
A. threshold of 2nd wave aggregation

B. initial rate of aggregation

C. % maximum aggregation

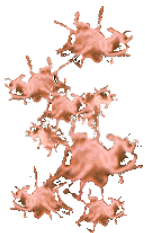
D. % final aggregation

- Shape change, length of lag phase, slope of aggregation, maximal amplitude or % aggregation, amplitude or % aggregation at the end, deaggregation, visual tracing, presence of 'secondary wave'
- Completed more than 4 h after collection: comment
- Establish appropriate reference intervals (SSC/ISTH, 2013)



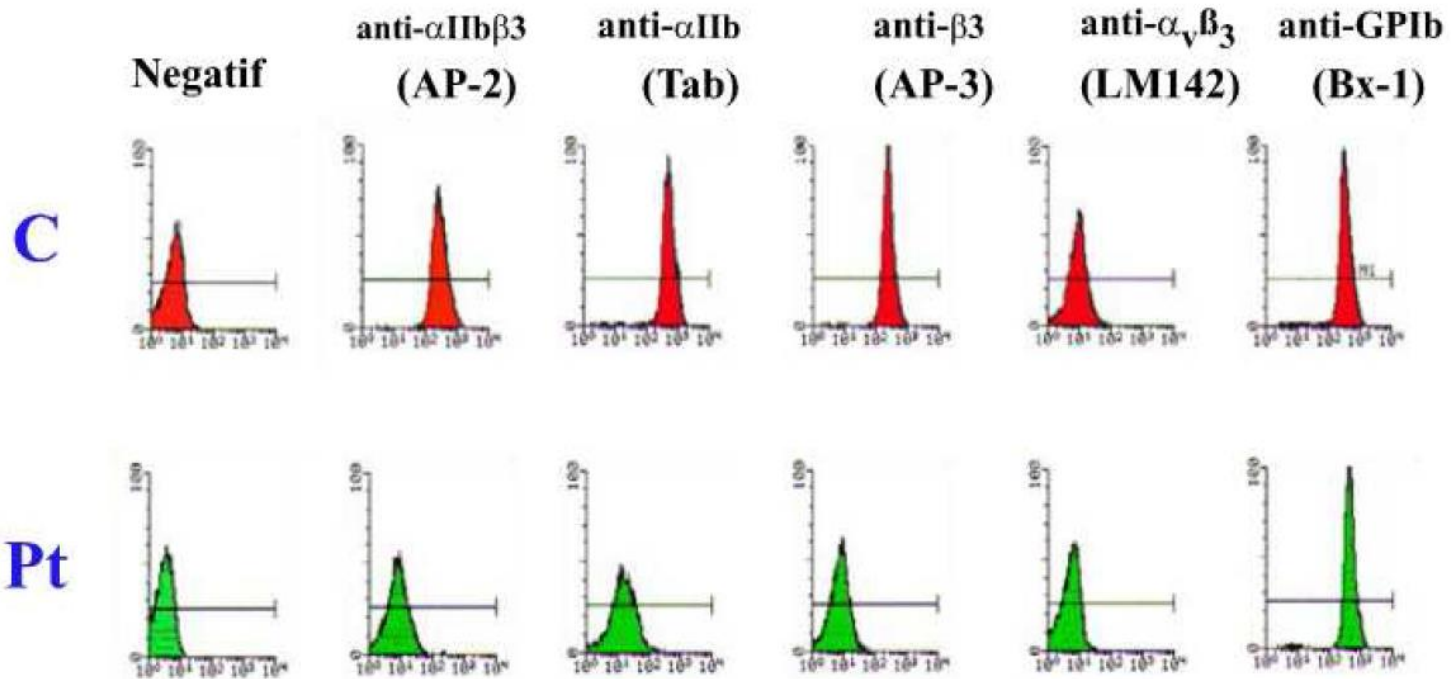
Flow Cytometry

- On whole blood or PRP
- Small volume of blood
- Large panel of antibodies
- Direct or indirect immunofluorescence
- Single or double staining
- Intact or permeabilized cells

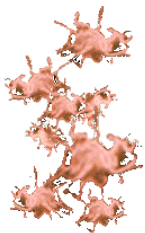


Flow Cytometry

Glanzmann Thrombasthenia diagnosis FACScan



β 3 Cys542Arg homozygote mutation



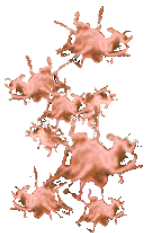
Flow Cytometry

- Most common activation markers
 - P-selectin expression (α -granule secretion)
 - Conformational change in integrin α IIb β 3 into active state (measured by PAC-1)
 - Platelet-leukocyte conjugates
 - Phosphorylation of VASP (P2Y₁₂ receptor activation)

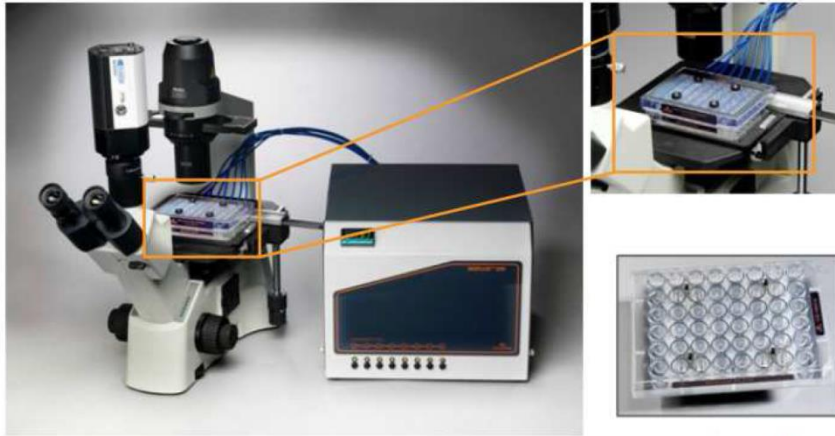


Platelet Adhesion in Flux Conditions

- So far, used only in very specialized laboratories
- Devices now available
- Information on spreading and thrombus formation at different shear rates

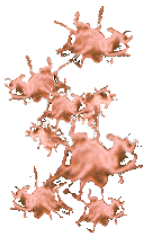


Platelet Adhesion in Flux Conditions



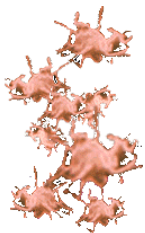
Flow Microchamber
(BioFlux-Labtech)

T-TAS (Total
Thrombus-
formation
Analysis System

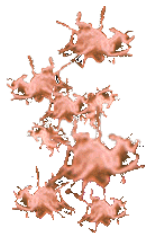
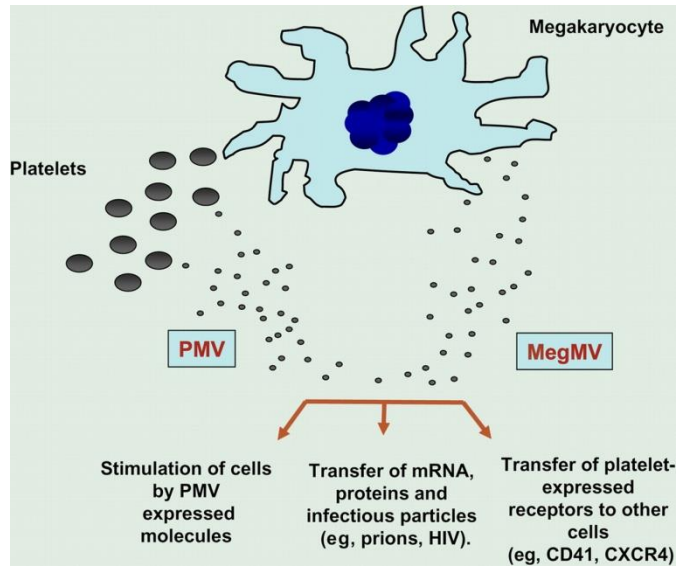
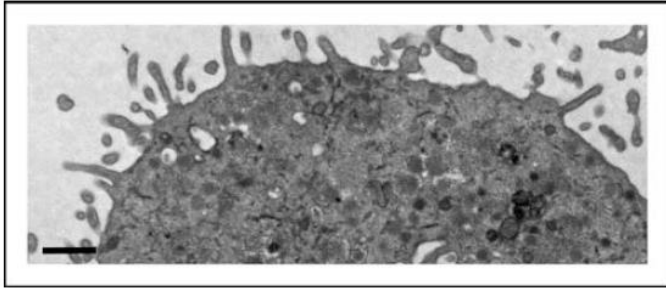


Platelet Microparticles (PMP)

- Small (0.1-1 um diameter) anucleoid phospholipid vesicles released from different cells, platelets erythrocytes, leukocytes and endothelial cells
- PMP being the most abundant, 70-90% of all circulating MP
- Marked elevation in many disease states: Prothrombotic and inflammatory disorders (Autoimmune disorders, atherosclerosis, malignancies, infections, etc)

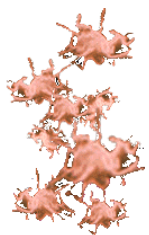
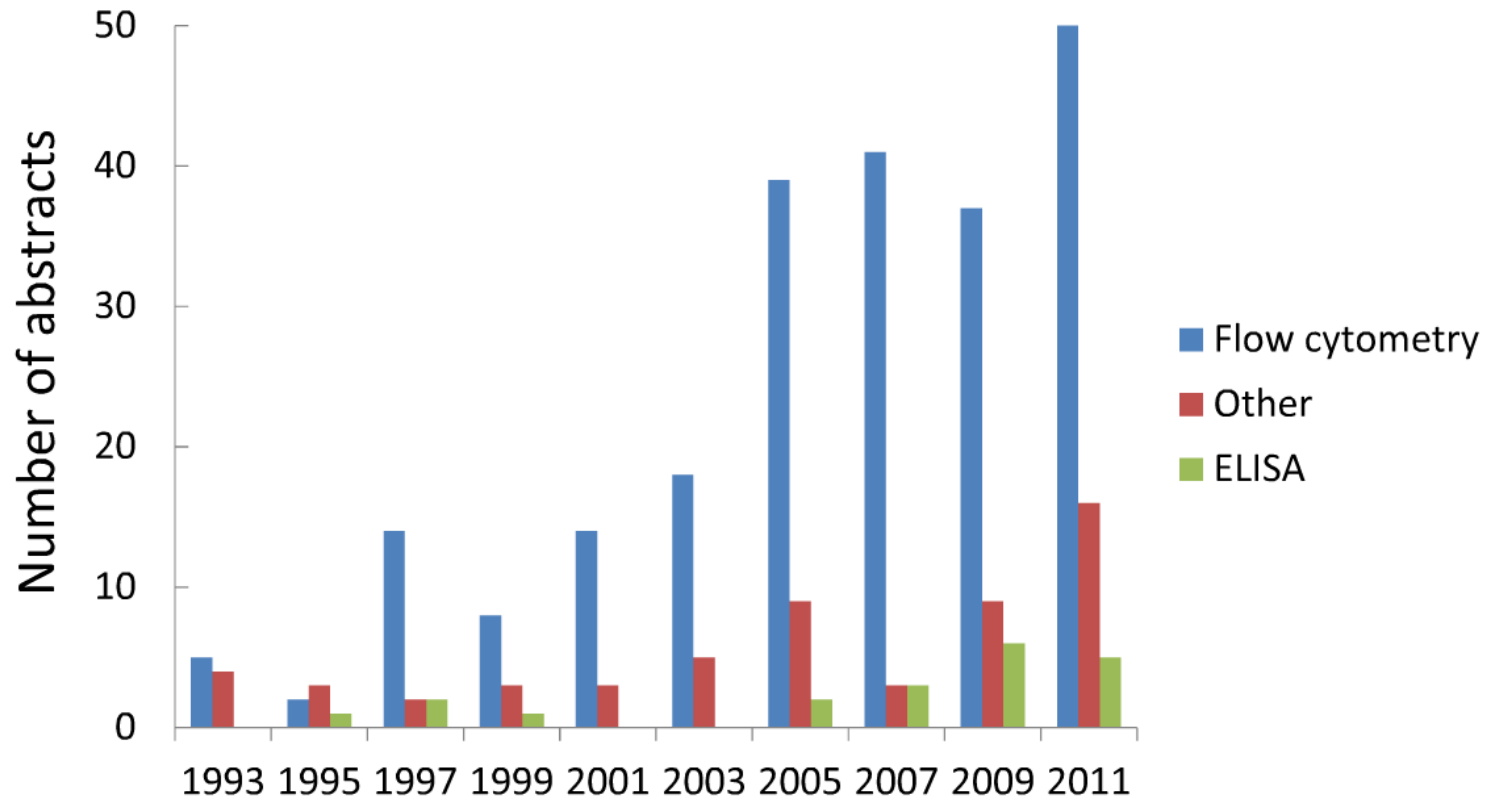


Platelet Microparticles



Feature	Exosomes	Microparticles	Apoptotic bodies
Size	50-100 nm	100-1,000 nm	Up to 4,000 nm
Sedimentation	100,000 g	20,000 g	16,000 g
Origin	Multivesicular, internal compartments	Plasma membrane	Cellular fragments
Release	Constitutive and/or cellular activation	Cellular activation and early apoptosis	Terminal apoptosis
Annexin V binding capacity	No/low	High	High
Marker proteins	Tetraspan protein CD63	Integrins, selectins, other antigen of parental cell	Histones

Platelet Microparticles

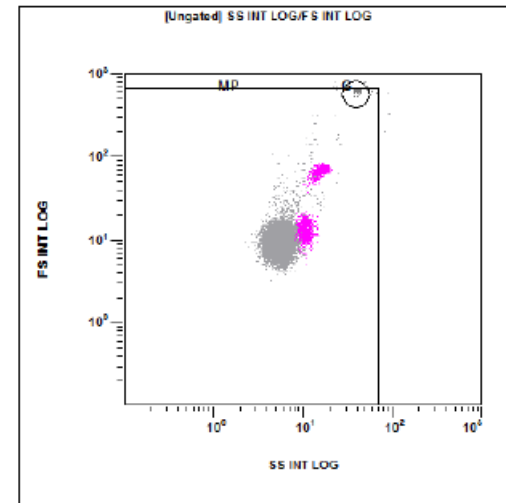
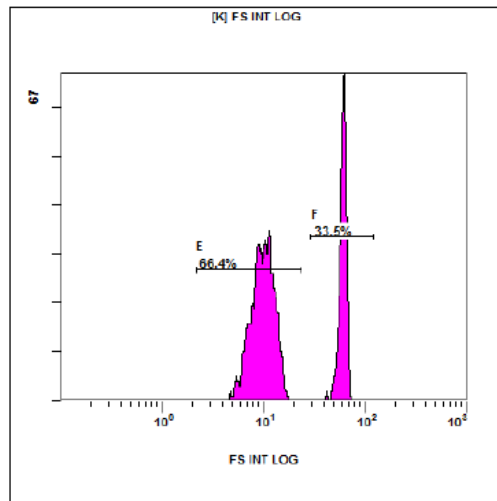


Platelet Microparticles

ISTH SSC Vascular Biology Workshop 3

Standardization of Microparticle Enumeration Across Different Flow Cytometry Platforms

~ Overall 36/46 (78.3%) Labs Qualified



[K] FS INT LOG

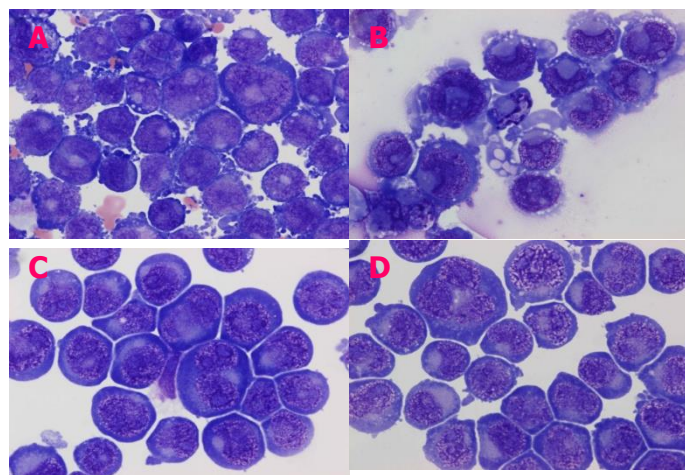
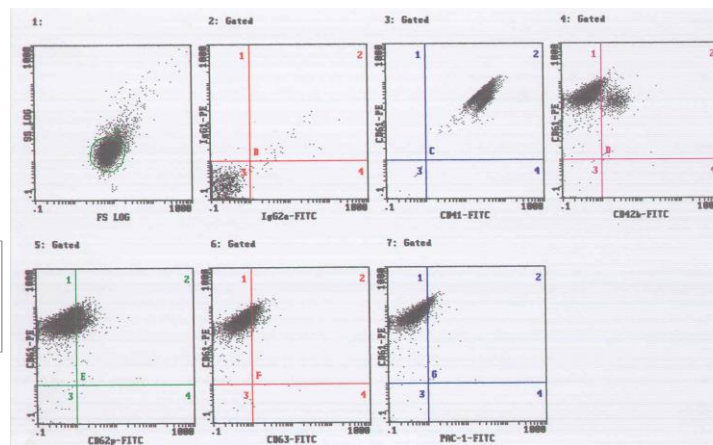
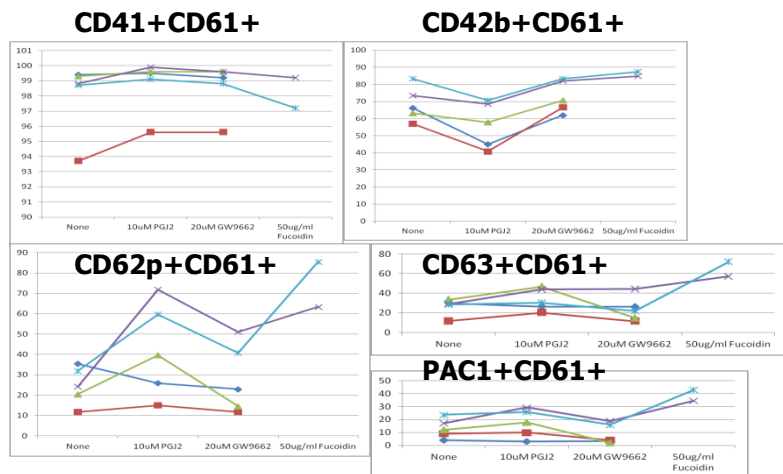
Region	Number	%Total	%Gated	X-Mean	HP X-CV	Y-Mean
ALL	4077	80.65	100.00	26.9	0.34	###
E	2707	53.55	66.40	10.1	1.37	###
F	1365	27.00	33.48	60.2	0.34	###

[Ungated] SS INT LOG/FS INT LOG

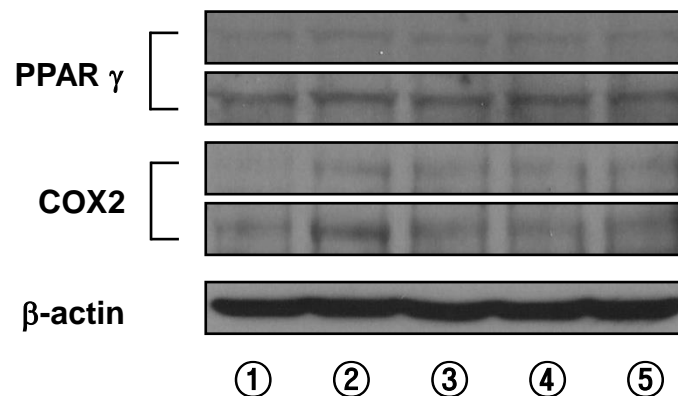
Region	Number	%Total	%Gated	X-Mean	HP X-CV	Y-Mean
ALL	26757	100.00	100.00	7.52	3.63	30.1
G	641	2.40	2.40	42.3	0.50	642
MP	26710	99.82	99.82	7.38	3.63	28.5



Signal Transduction



①	None	24h incubation
②	10 μ M PGJ ₂	
③	20 μ M GW9662	
④	50 μ g/ml Fucoidan	
⑤	25 μ g/ml Seanol	



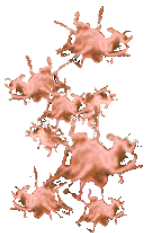
Future Directions

- Many challenges in ensuring accurate and meaningful results
 - Many remain poorly standardized
 - No widely available internal and external quality control materials and programs
 - Specimen quality, anticoagulation, reference ranges, etc.



Future Directions

- Genomics and proteomics are powerful tools for studying platelets.
 - Gene screening for known variations
 - Direct sequencing of promoters, exons and splice sites
 - Whole exome sequencing
 - Proteomics allows a large-scale study of the platelet proteome.



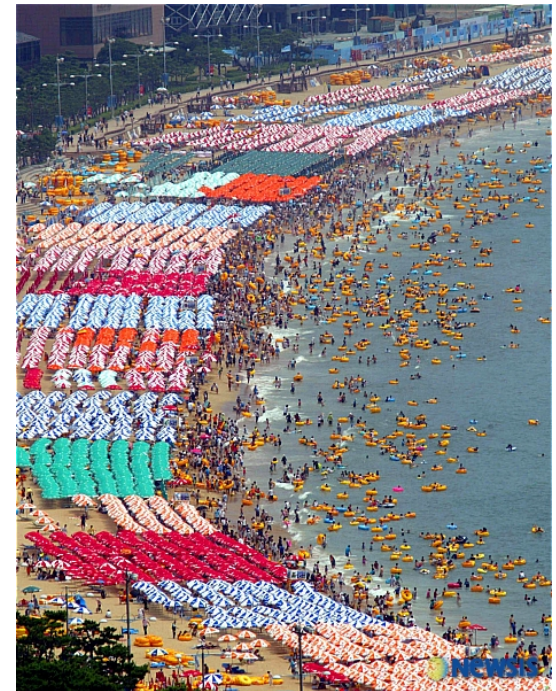
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- <http://www.ascp.org/>
- <http://www.islh.org>
- <http://www.isth.org>



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