Immunologic and Hemostatic Effects of Cold Storage of Platelets

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THOR 2019 RDCR Conference

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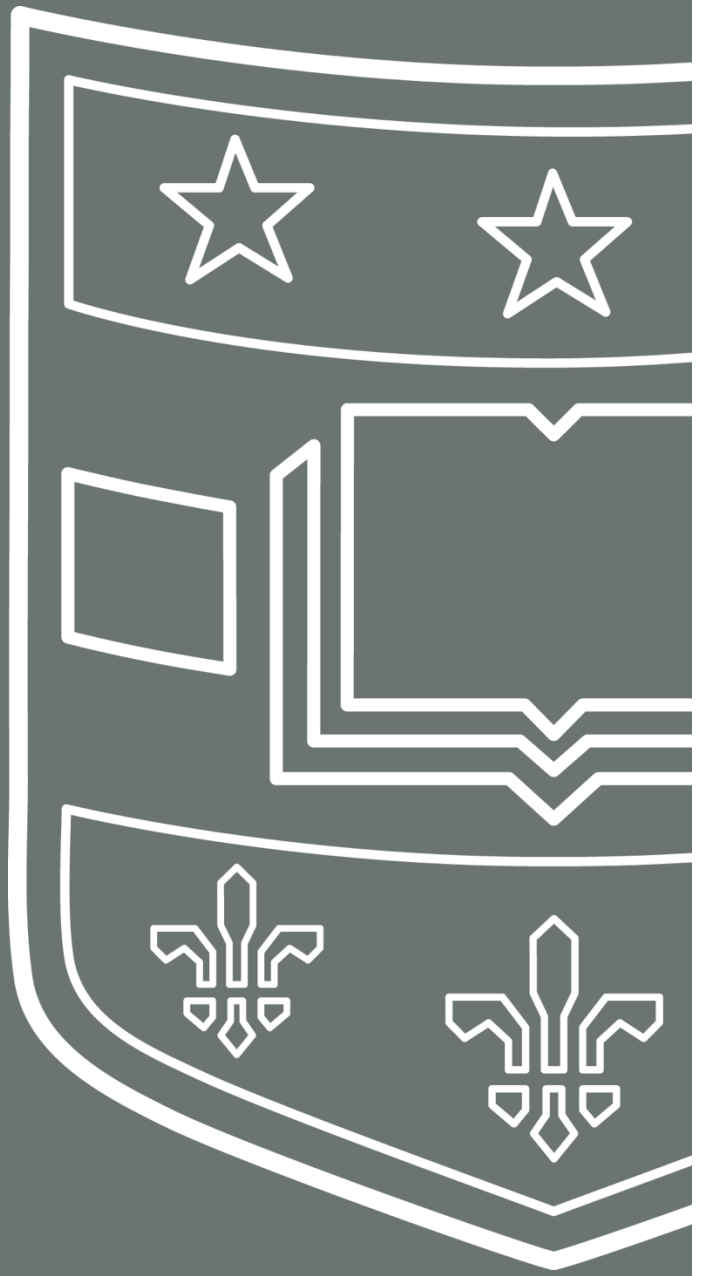
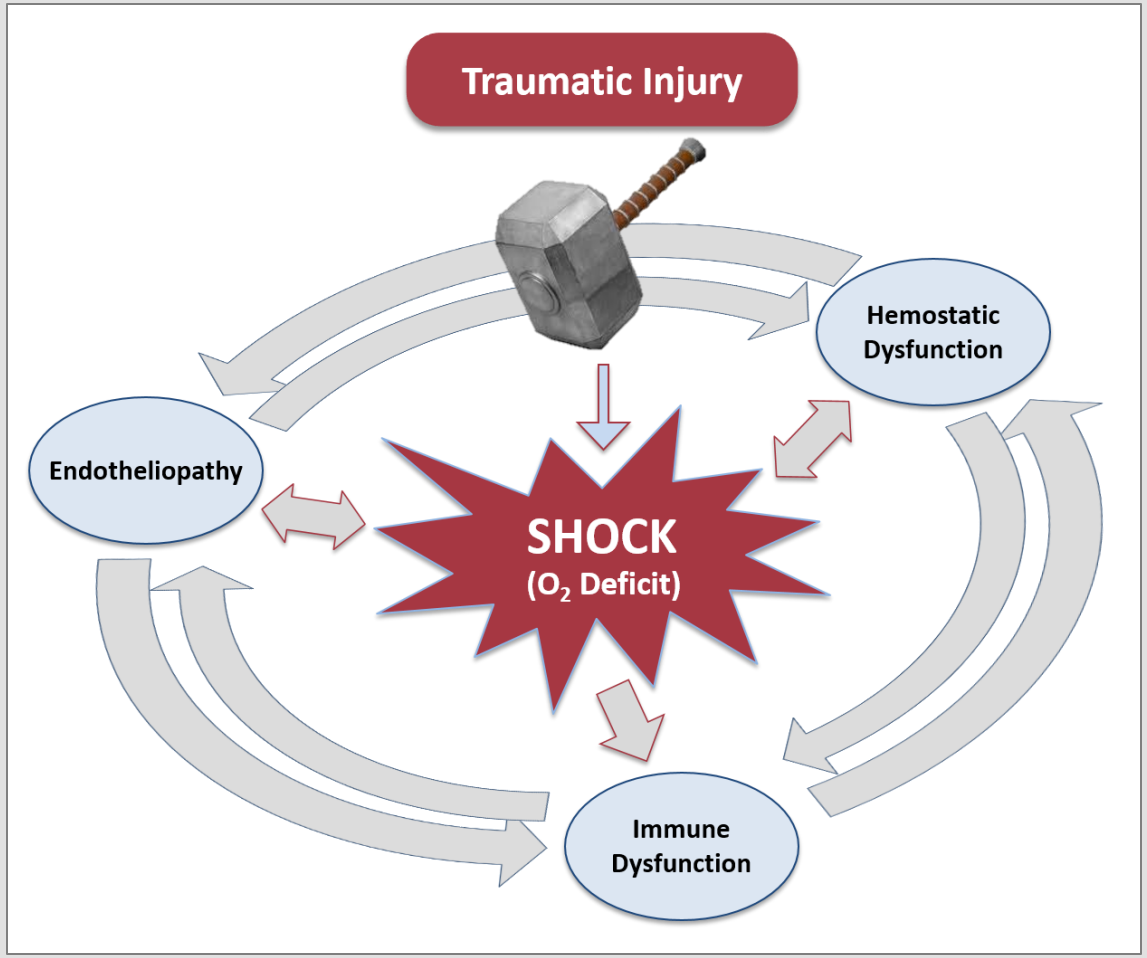


Washington University in St. Louis

Trauma-Induced Blood Failure



- Shock
  - Hemostatic dysfunction
    - Trauma-induced coagulopathy (TIC)
  - Immune dysfunction
    - Global inflammation = MODS
    - Immune specific = increased risk of infection
  - Endotheliopathy
    - Glycocalyx and endothelial cell damage biomarkers correlate with mortality

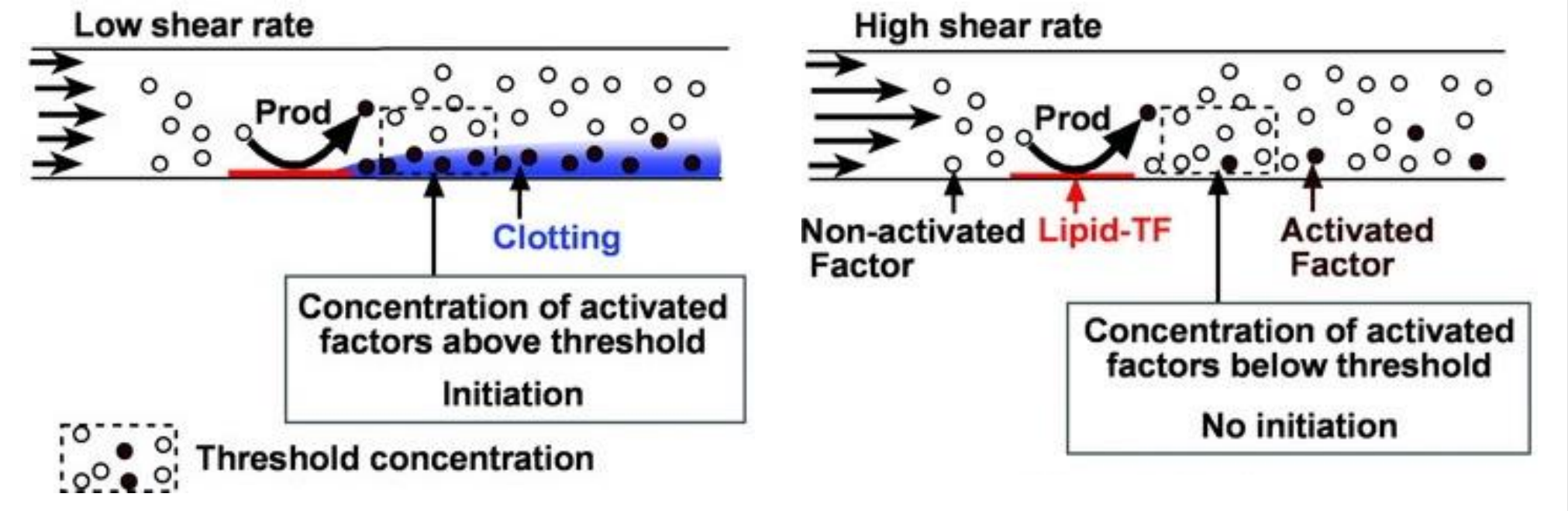
Cold-Stored Platelets: Benefits

- Damage Control Resuscitation – importance of platelets
- Cold-stored platelet benefits
  - ▲ hemostatic function *in vitro*
  - ▼ bacterial contamination
  - ▲ shelf life
  - ▼ wastage and cost
- Sparse *in vivo* evidence of hemostatic function – 1973 to Norwegian trial
- Better understanding of potential multi-faceted effects of cold-stored platelet transfusion

Cold-Stored Platelets: Function?

- More development and basic research re: cold-stored platelets
  - Hemostatic: reductionist methodologies (no flow, single agonists)
  - Immune and Endothelial: no methodologies
  - Lack of translatability to *in vivo* function
- Microfluidic assays
  - Flow (arterial and venous shear)
  - Biological surfaces (collagen and tissue factor)

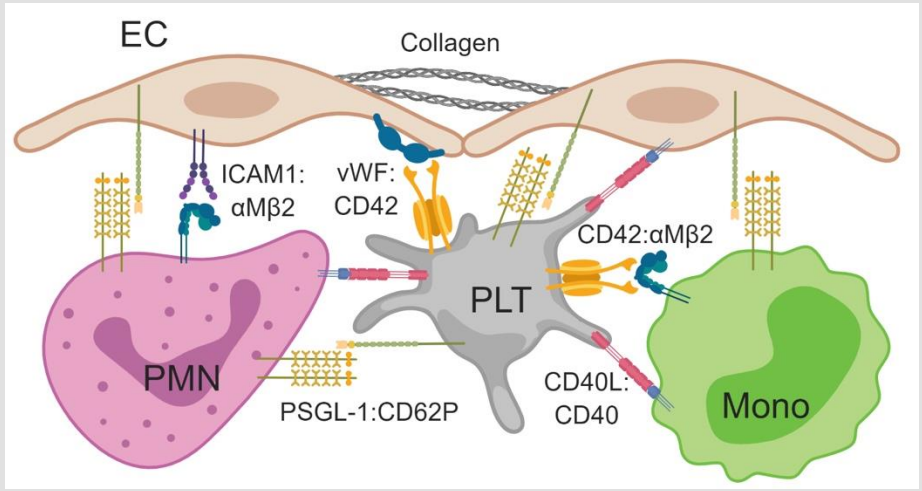
Shear Flow and Platelet Function and Hemostasis

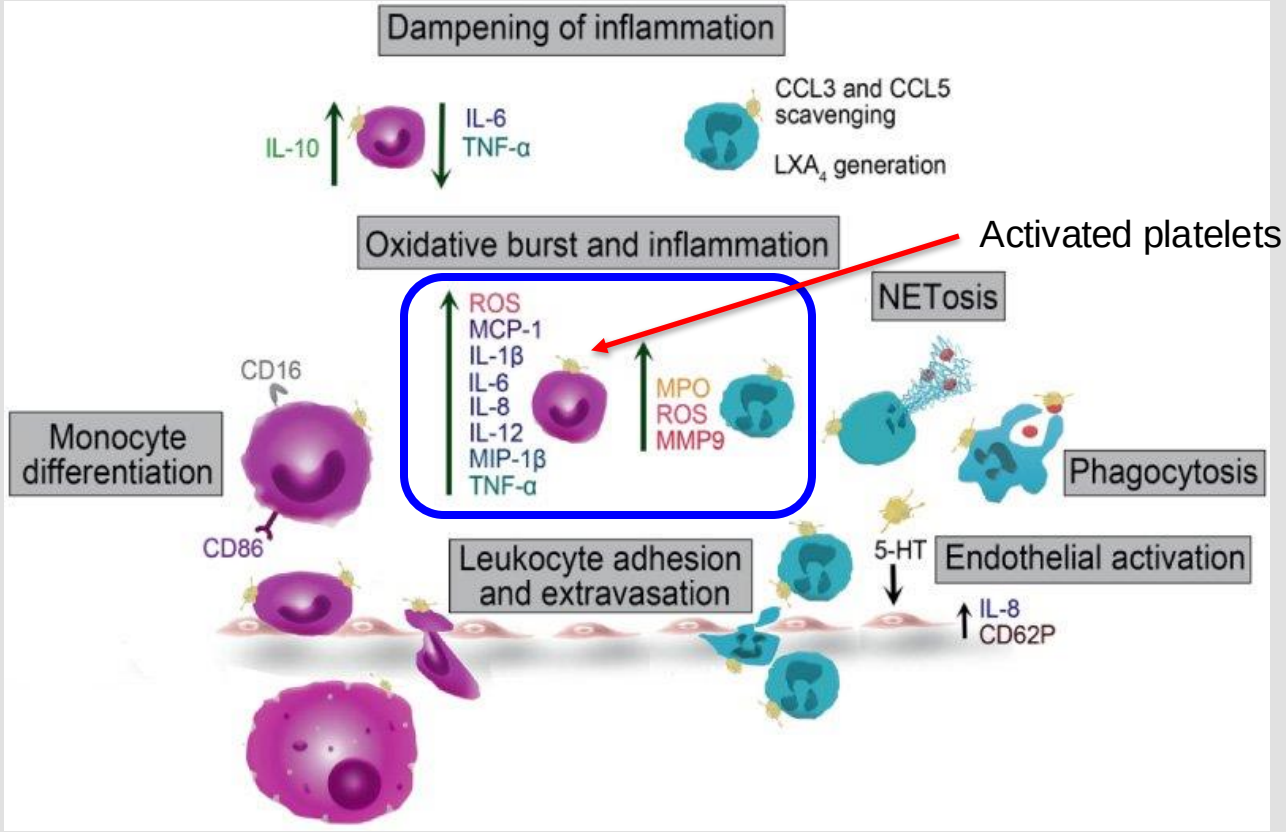


- Importance of flow
  - Low Shear (venous) – coagulation proteins, diffusion-dominated → fibrin-rich clot
  - High Shear (arterial) – vWF elongation, convection-dominated → platelet-rich thrombi
- **How does cold storage of platelets alter their function in *in vivo* flow-dependent physiologies?**

Adapted from PMID:18703776

Platelets: Mediators of Hemostasis and Immune Crosstalk



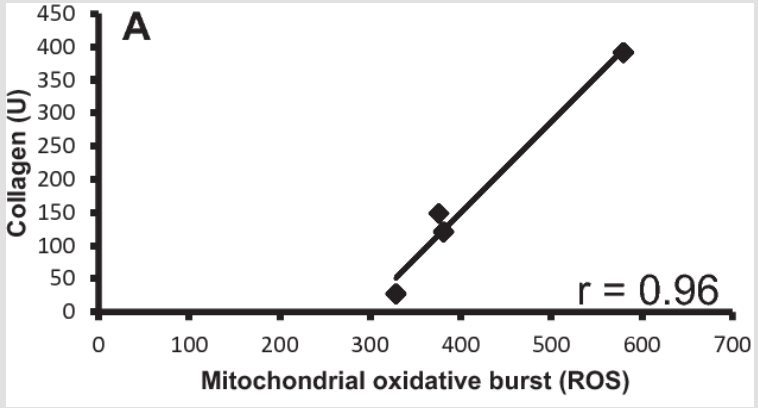


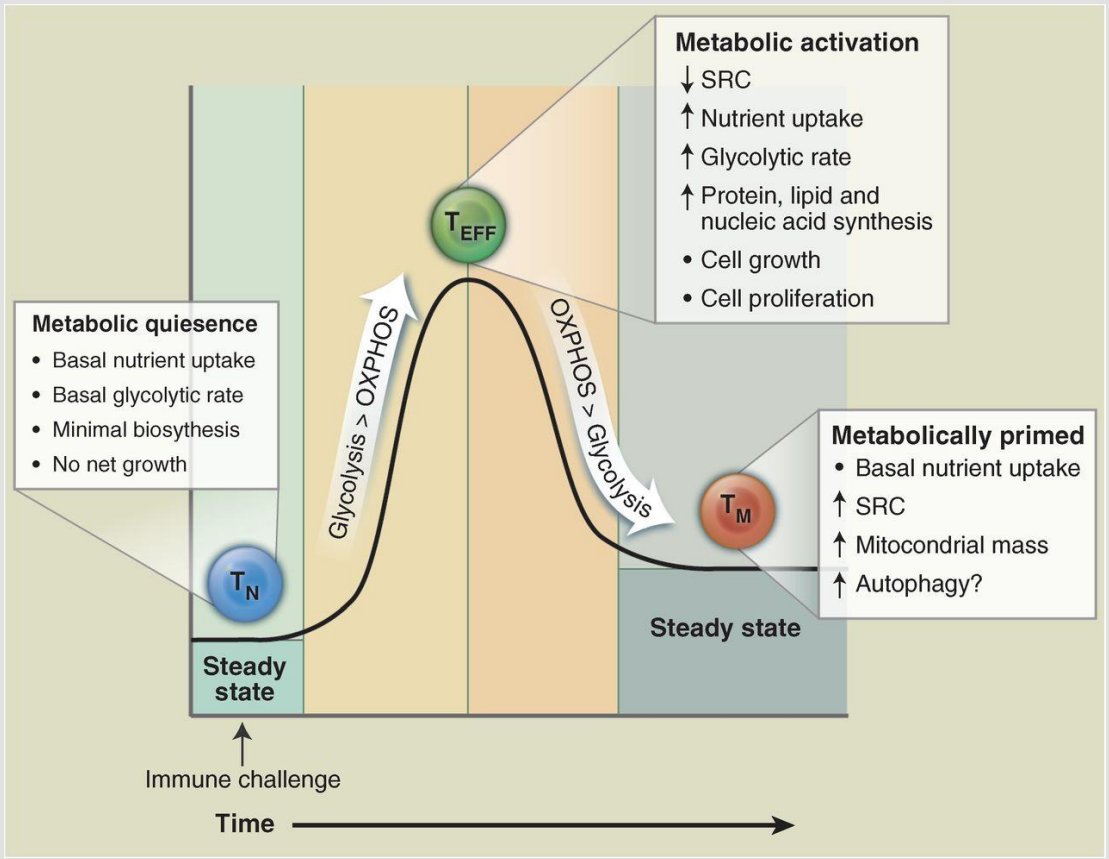
- **How does cold storage of platelets alter their function in *in vivo* immune processes?**

Adapted from PMID:27226790

Metabolism Informs Function

	Baseline	RT		4°C	
	Day 1	Day 3	Day 5	Day 3	Day 5
pH	7.24 ± .07	7.45 ± .16	7.28 ± .08	7.45 ± .21	7.44 ± .13
Lactate, mg/dL	1.77 ± 0.6	6.85 ± 0.8*	12.87 ± 2.1*	4.51 ± 0.6*	5.67 ± 0.3*†
Glucose, mg/dL	321.5 ± 8.1	294.0 ± 22.1*	238.3 ± 42.5*	310.2 ± 14.2*	307.5 ± 25.0*†





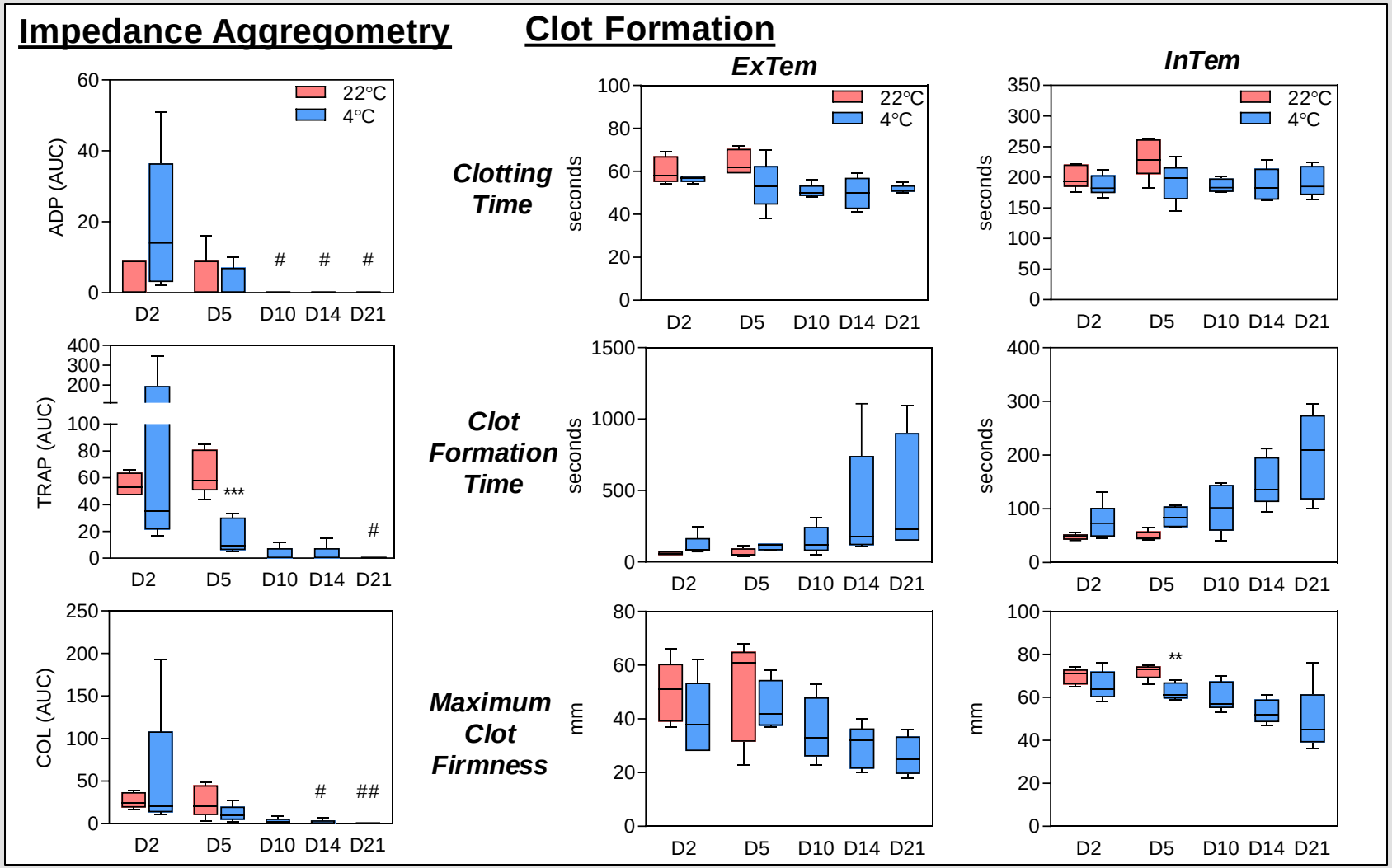
Top: adapted from PMID:24169210; Bottom: adapted from PMID:27001365

PMID: 24115444

Study Design and Objectives

Apheresis Units	Hemostasis Time Points	Hemostasis Assays Performed	Immune Time Points	Immune Assays Performed
22°C-PLT *+ agitation*	D2, D5	• Platelet counts • Impedance aggregometry • ROTEM ExTem, InTem • Fibrinogen levels • Thrombin generation • Microfluidic perfusion	D0, D5	• Metabolic Profiling • Surface Staining • Respiratory Burst Induction • Cytokine Induction
4°C-PLT *+ agitation*	D2, D5, D10, D14, D21		D0, D5, D10, D21	

Aggregation, not Clot Formation, is Impaired by D10 of Cold Storage



**, p<0.01, ***, p<0.001 when compared to warm at the same time point; #, p<0.05, ##p<0.01 when compared to D2 cold; n=5

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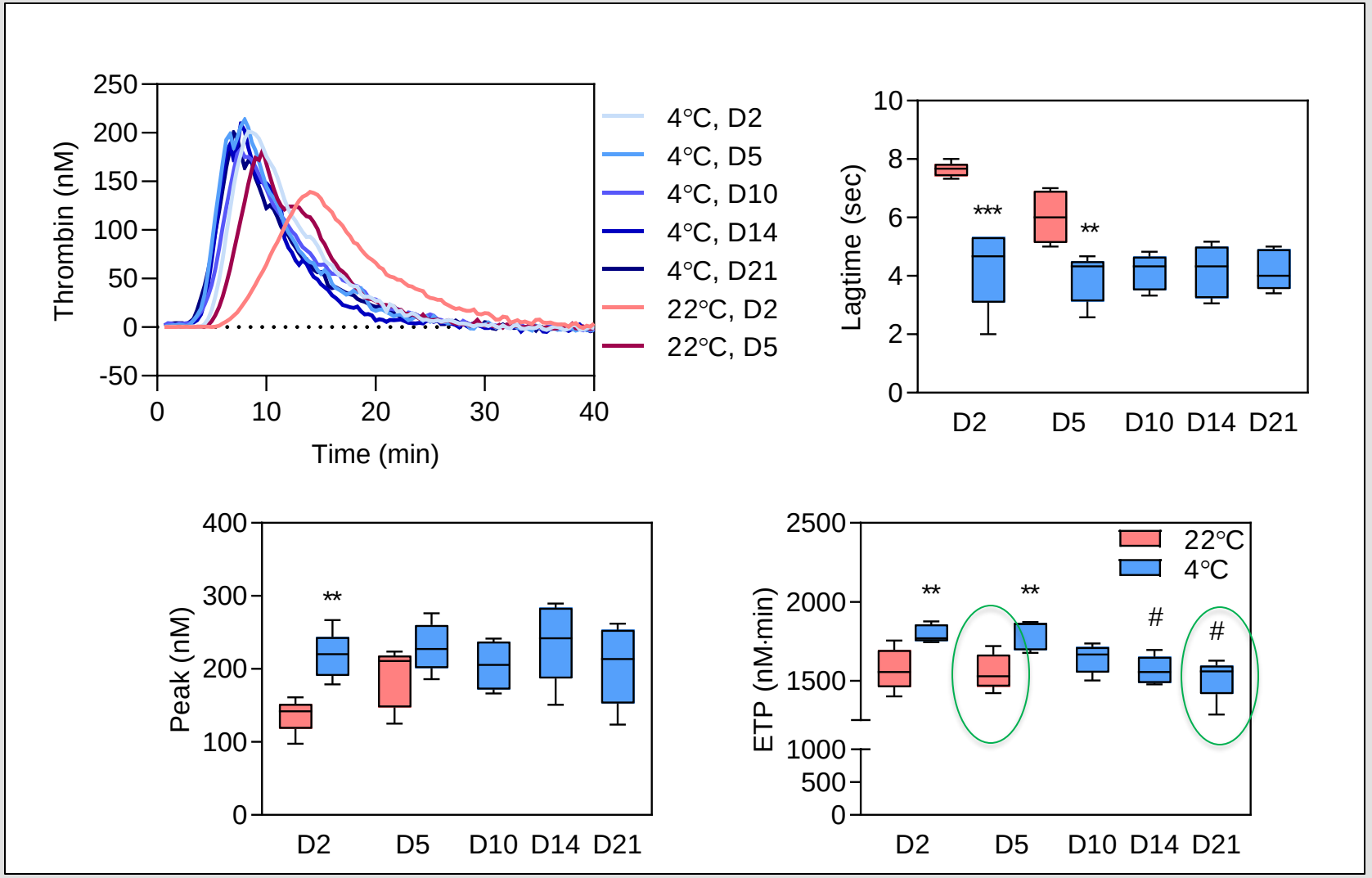
Cold Storage Preserves Thrombin Potential



**, p<0.01, ***, p<0.001 when compared to warm at the same time point; #, p<0.05, when compared to D2 cold; n=5

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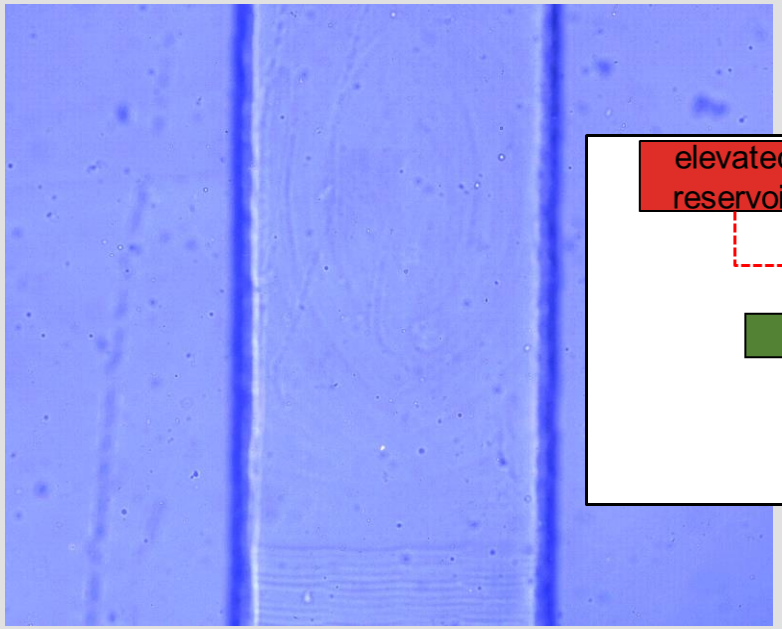
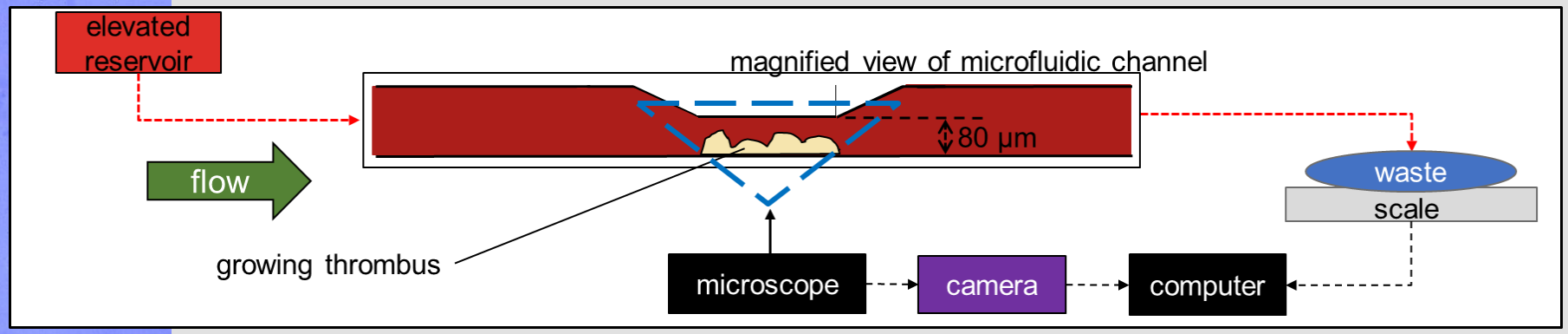
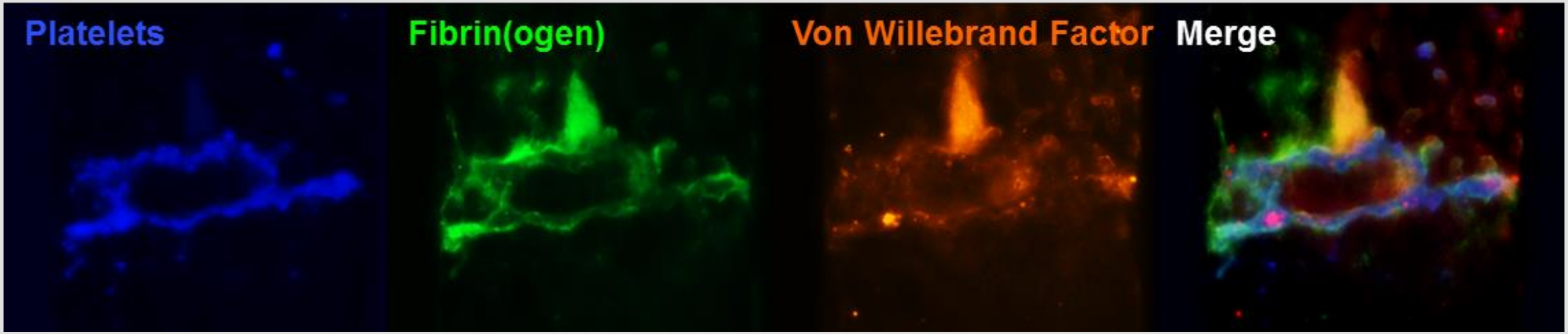
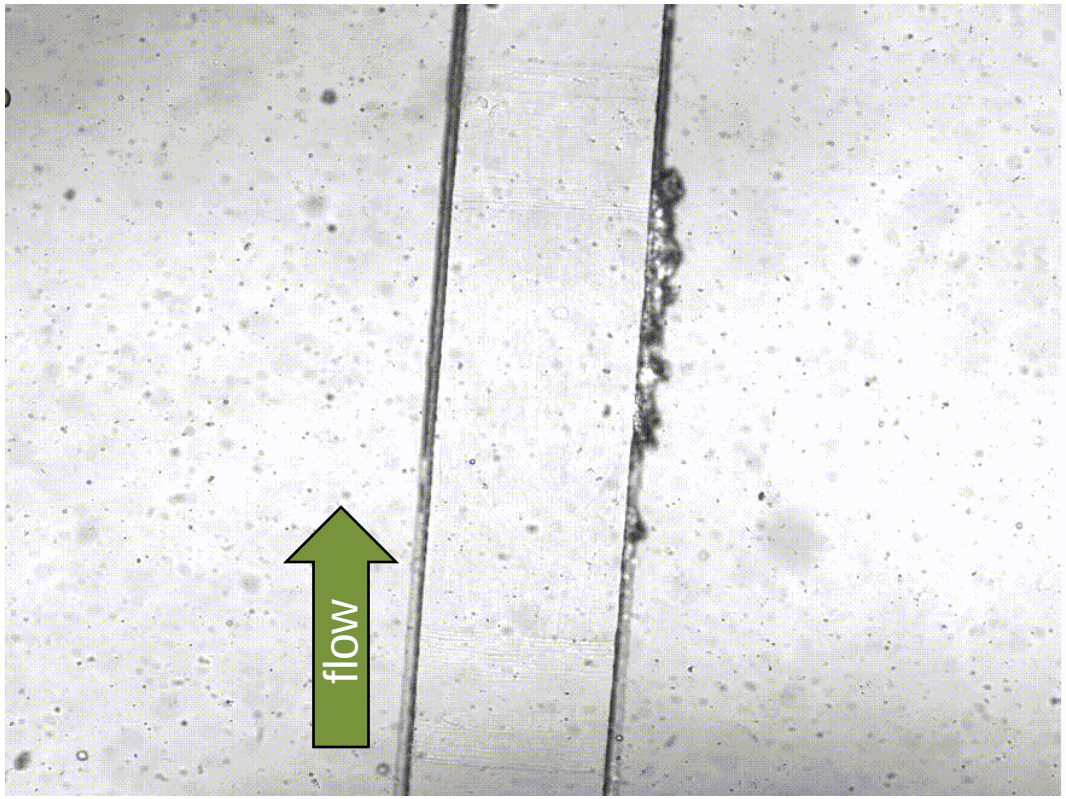
Microfluidic Assays

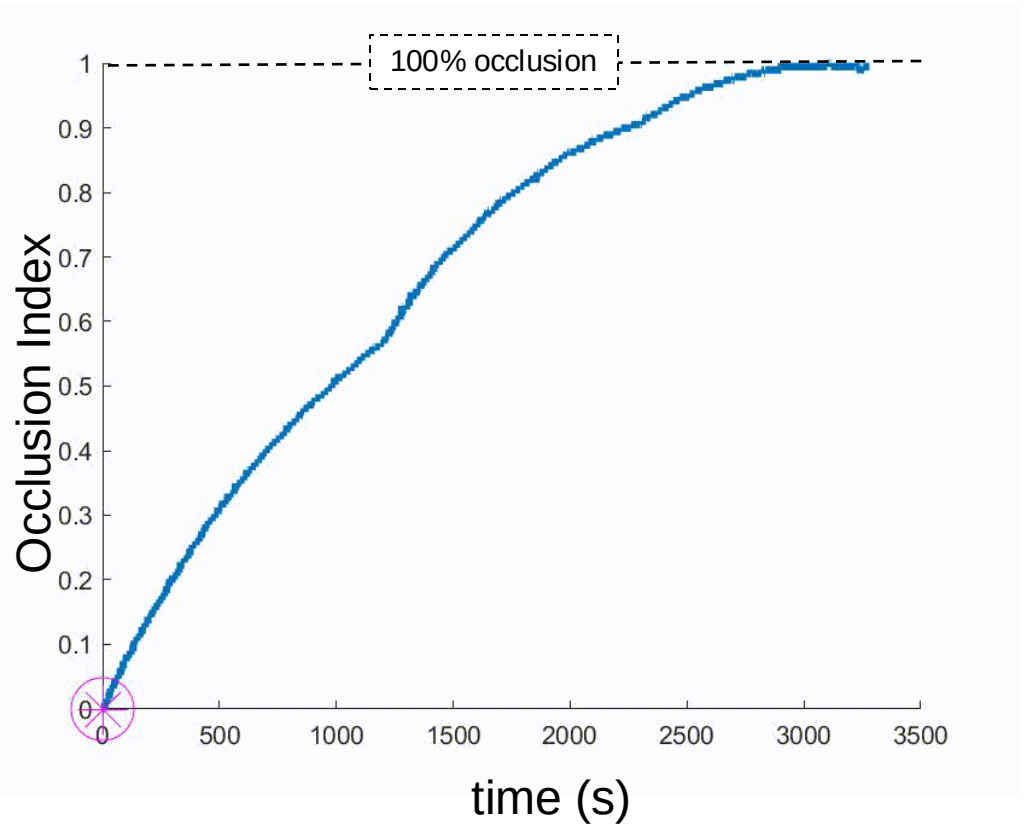




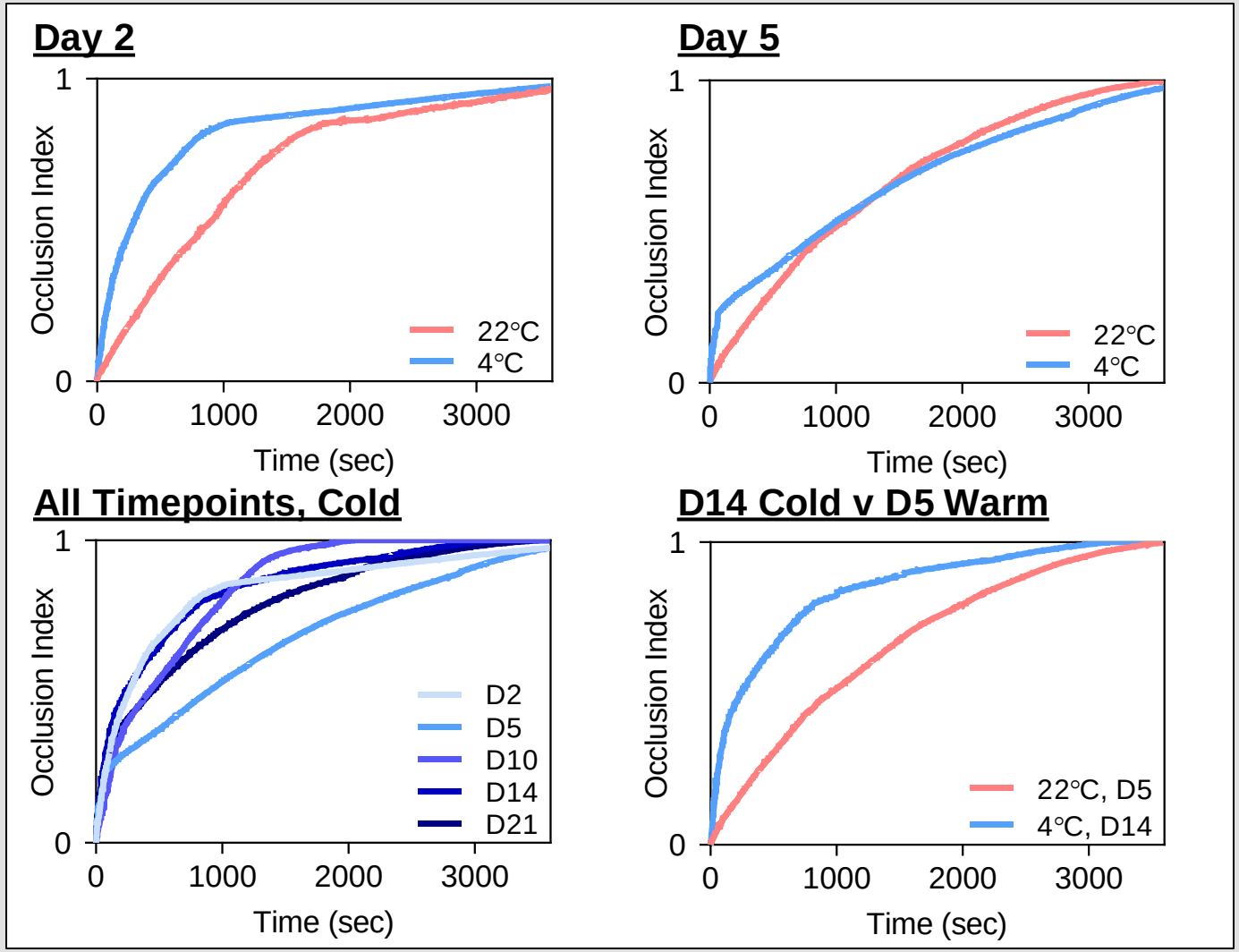


Microfluidic Chamber Occlusion





Occlusion Under Flow is Not Significantly Different Between Storage Conditions



n=5

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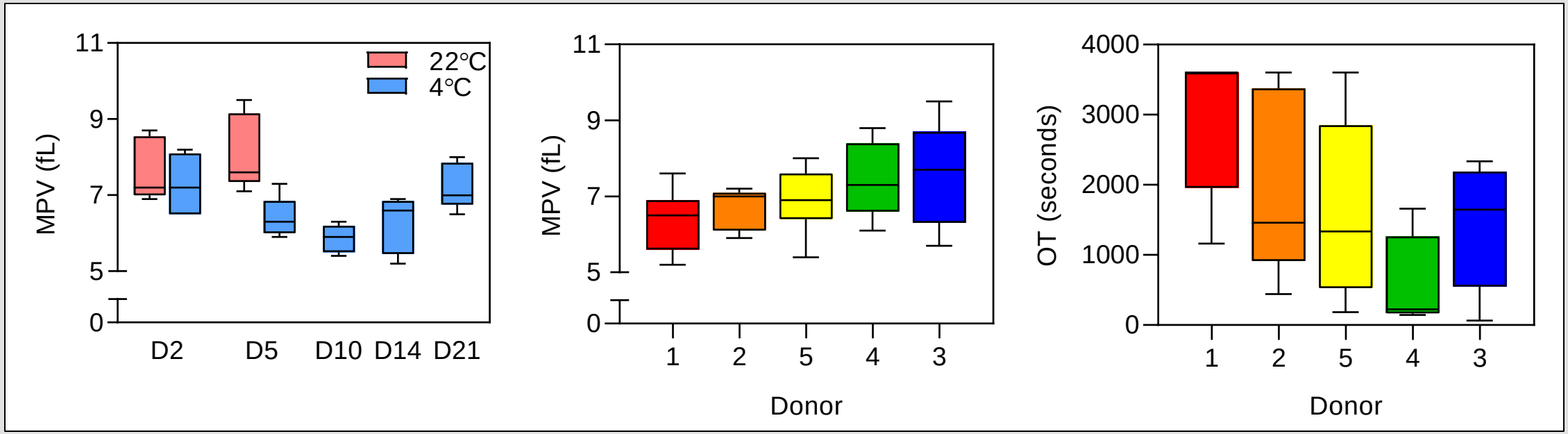
Donor Variation Impacts Occlusion Time



n=5

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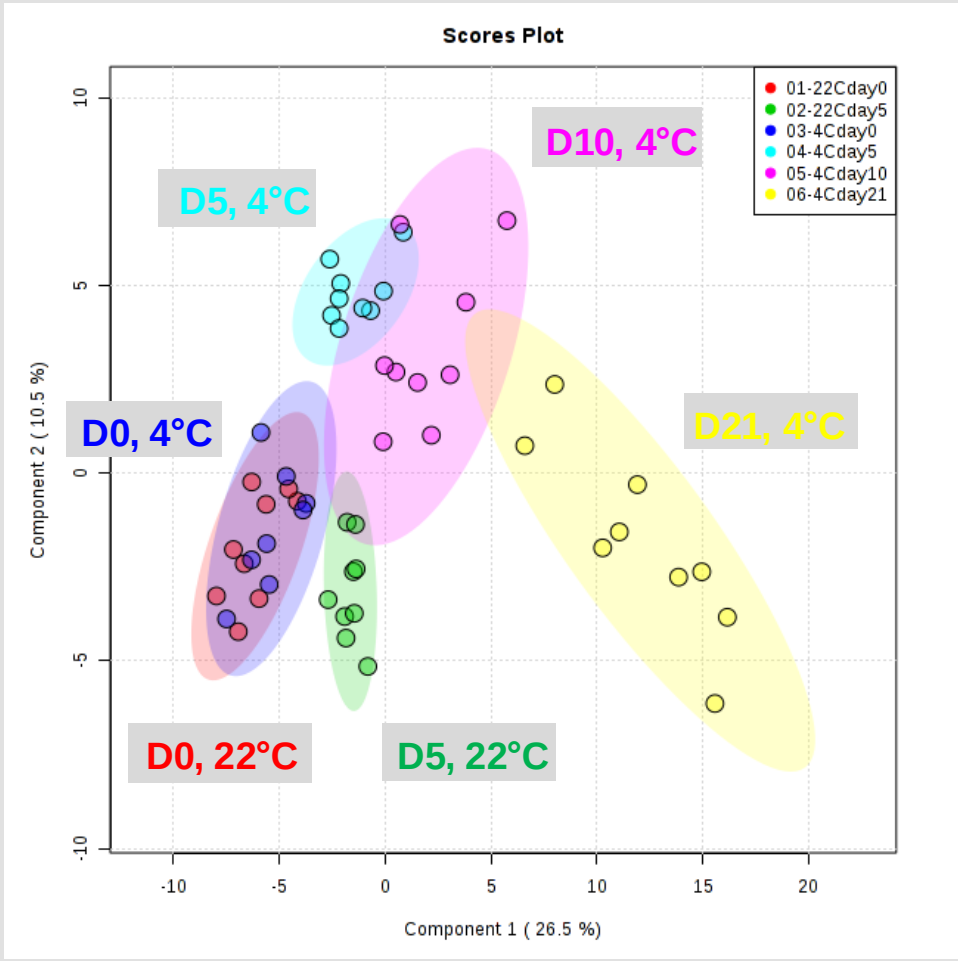
Hemostasis Summary

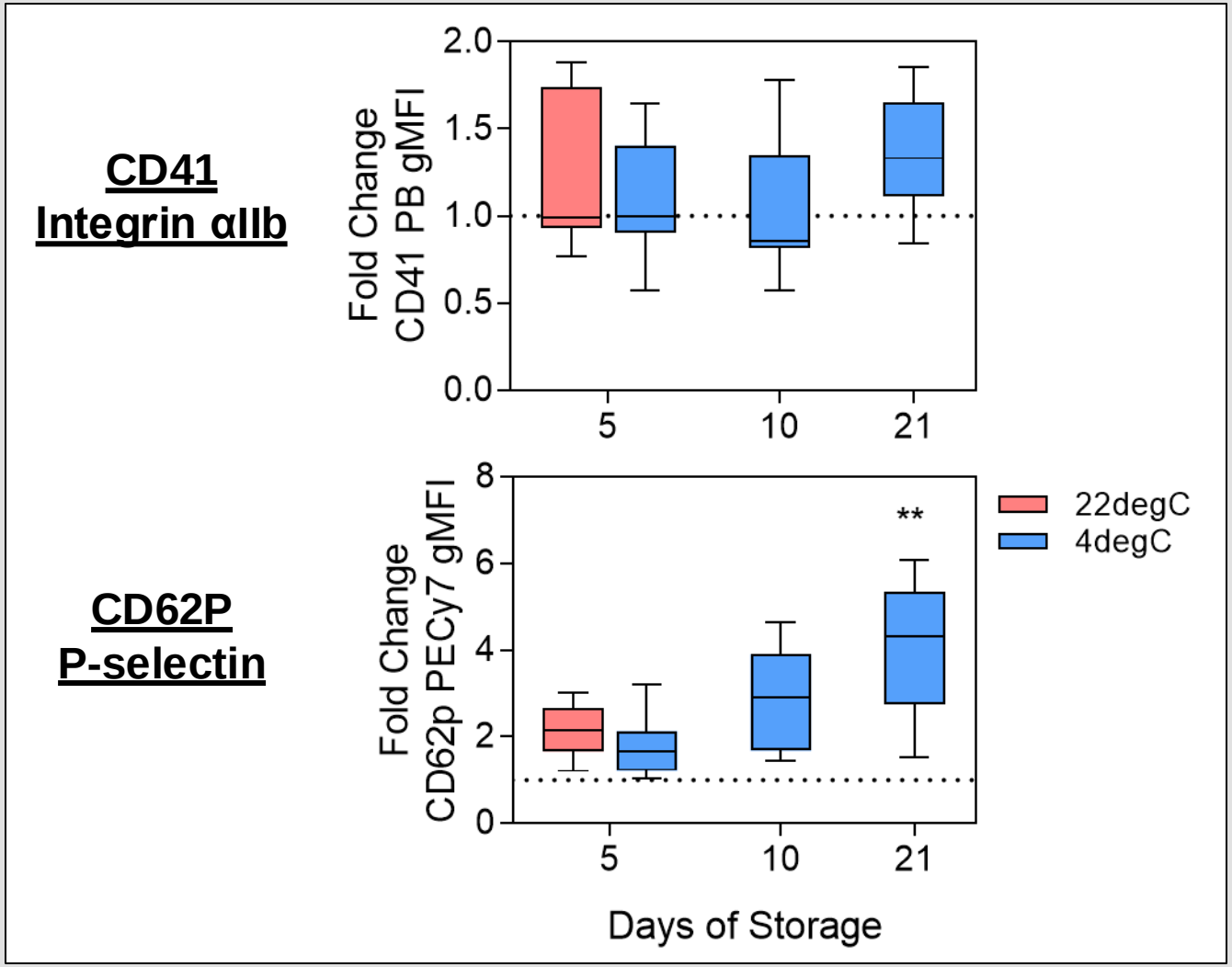
- Aggregometry assays deem cold stored platelets less effective by day 10 of storage when compared to D2.
- CT is preserved in viscoelastic assays, while CFT and MCF increase and decrease, respectively, over storage duration
- Cold storage preserves thrombin generation
- Microfluidic assays demonstrate cold-stored platelets are capable of clot formation on collagen in an arterial flow setting up to 21 days of storage
- Both occlusion time and MPV are donor-dependent, and are inversely related

Time over Temperature, Imparts the Largest Impact on Platelet Metabolism



n=9

Cold Storage Augments CD62P/P-selectin Expression

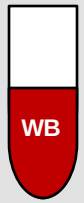


n=9

**, p<0.01 when compared to D5 within the same group – Kruskal Wallis

Platelet:Leukocyte Culture & Respiratory Burst

Respiratory Burst Assay



15 min

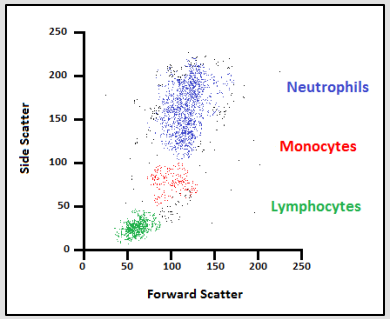
Load WB with DHR

45 min

± stimuli:
Media alone
OR
22°C PLT
OR
4°C PLT

Lyse RBC, Stain with CD107a

Flow Cytometry

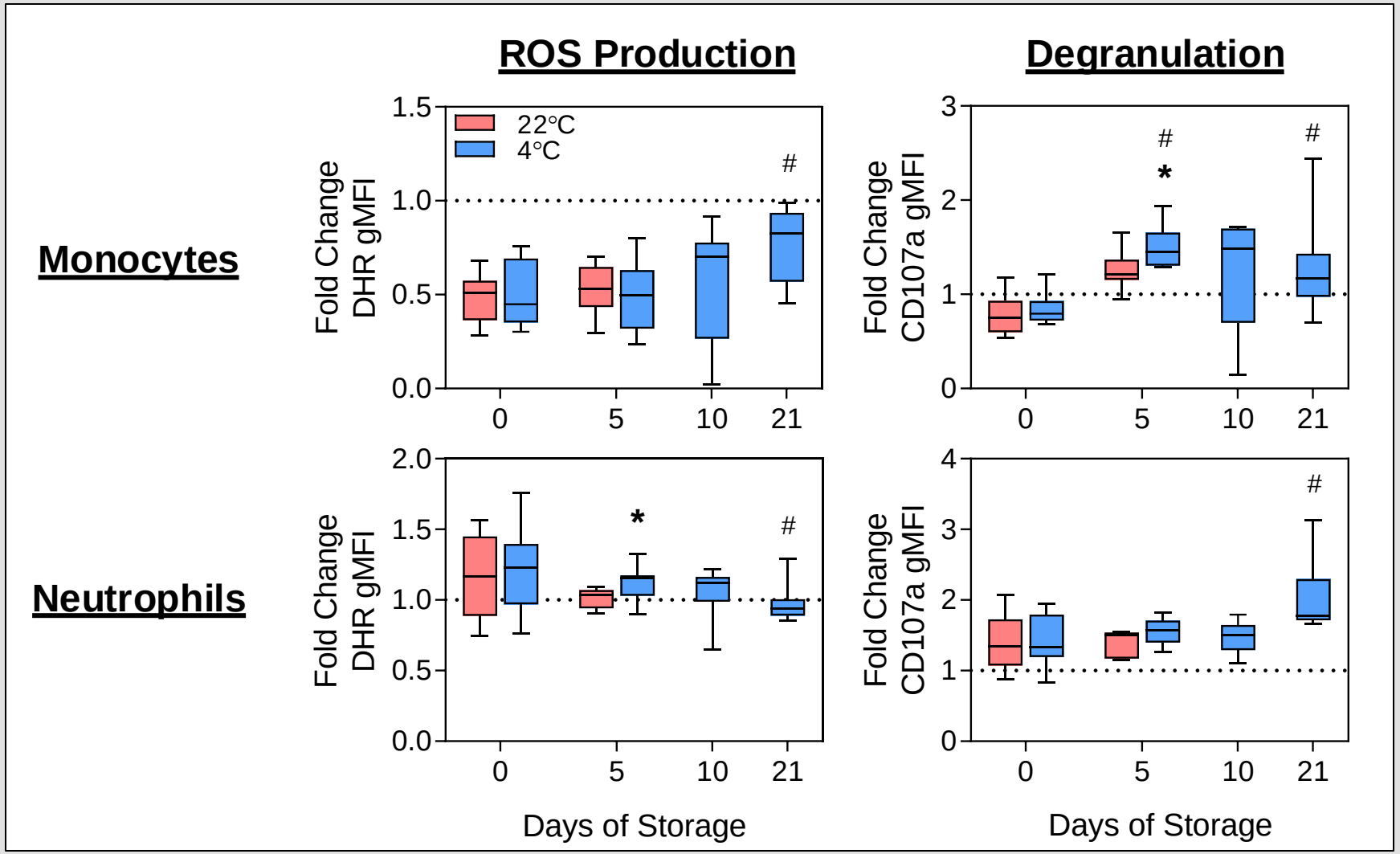


Identify if stored platelets:

- Induce respiratory burst in myeloid cells
- Cause myeloid degranulation

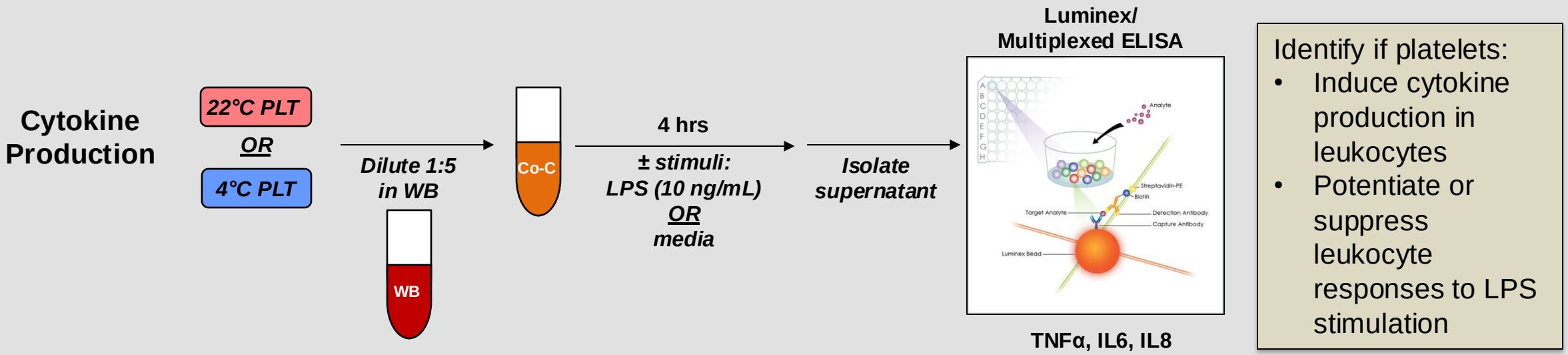
https://www.labome.com/method/Flow-Cytometry-A-Survey-and-the-Basics.html

Warm or Cold, Platelets Suppress Monocyte Burst



*, p<0.05 when compared to warm at the same time point; #, p<0.05 when compared to D0 cold; n=9

Platelet:Leukocyte Culture & Cytokine Induction

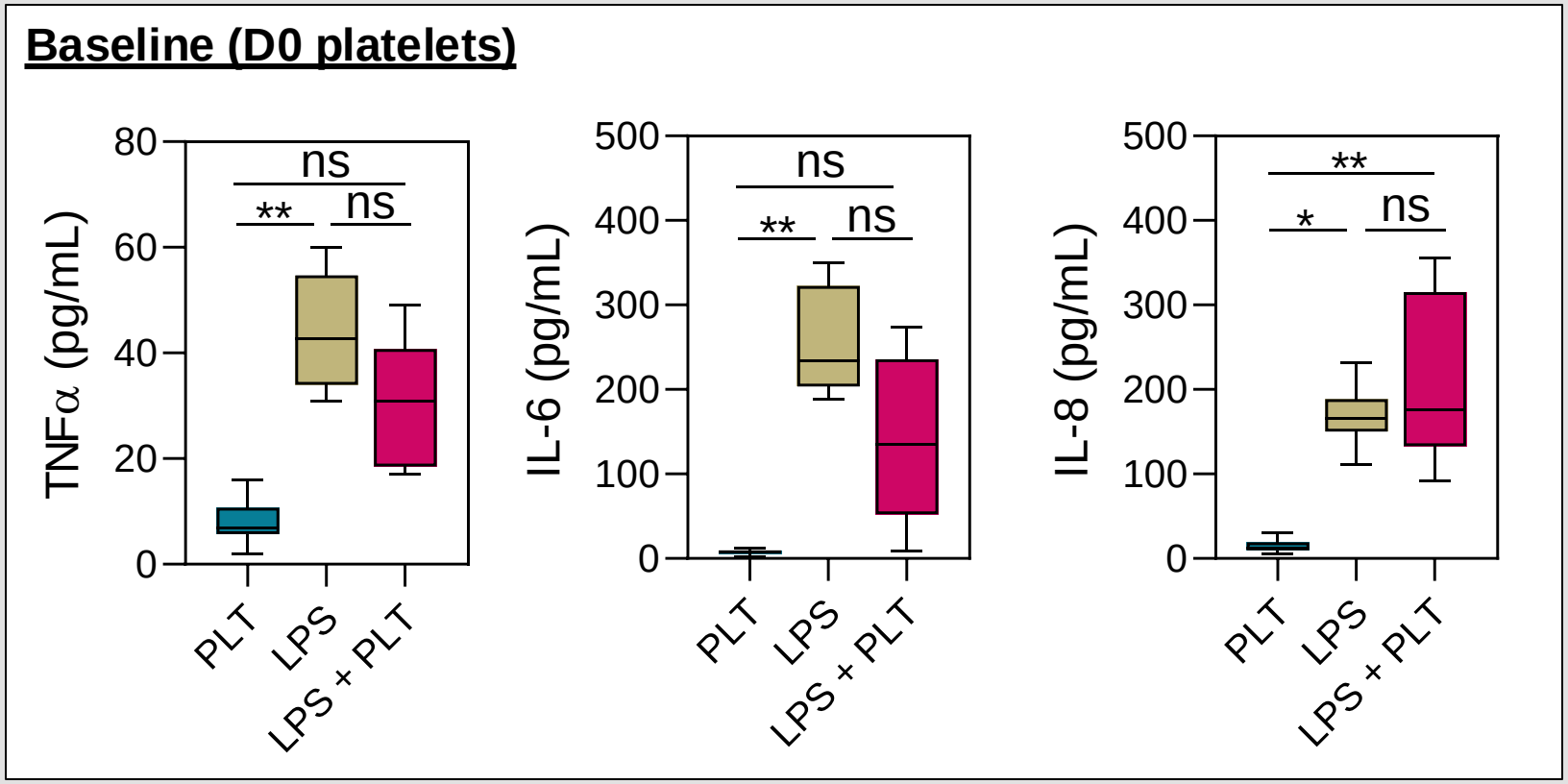


Identify if platelets:

- Induce cytokine production in leukocytes
- Potentiate or suppress leukocyte responses to LPS stimulation

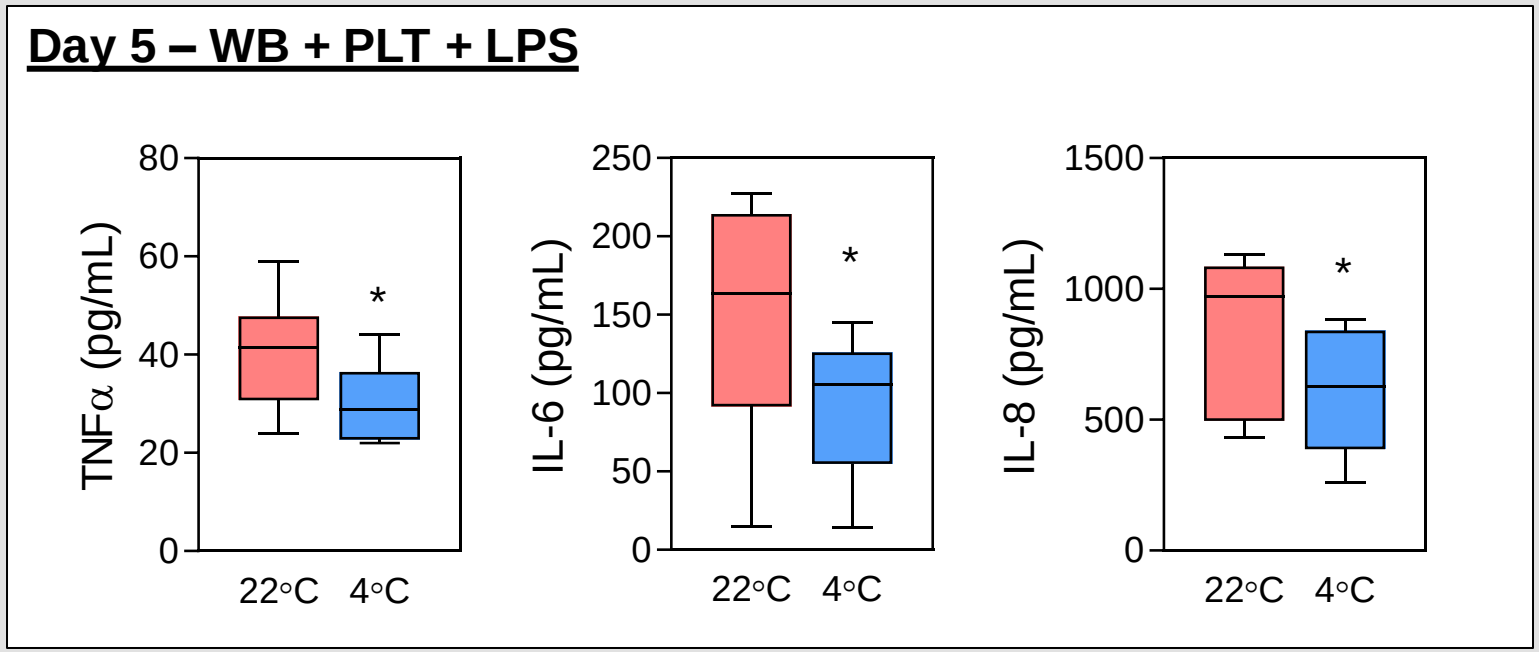
https://whitesci.co.za/brand/rnd-systems/luminex-immunoassay-guide/

Platelets Alone do not Induce Cytokine Production



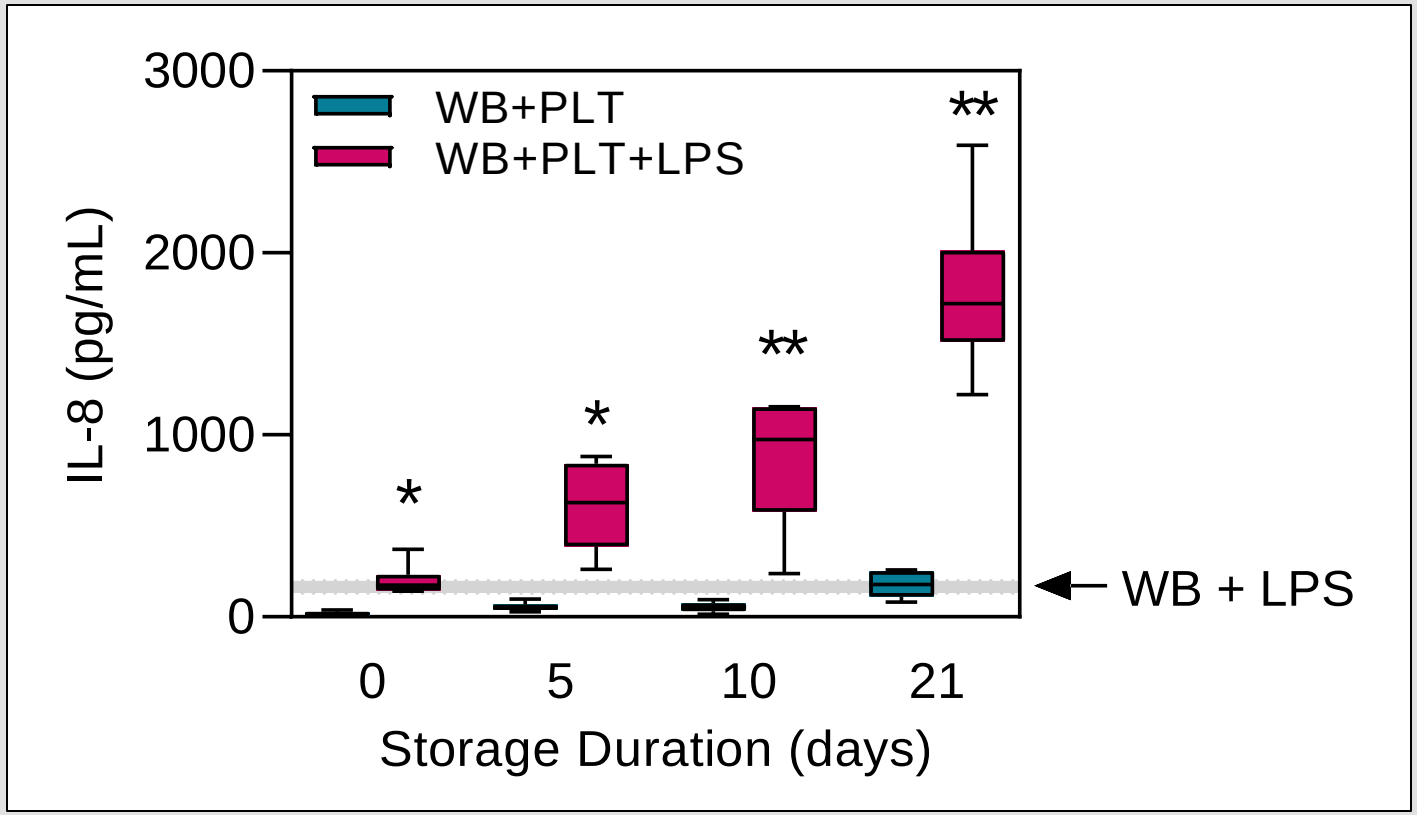
Day 0: ns, p>0.05, *, p<0.05. **, p<0.01; n=9

Cold Storage Reduces Platelet-induced Cytokine Production by Leukocytes



*, p<0.05 when compared to 22°C; n=9

Day 21 Cold Platelets Potently Induce IL-8 From Leukocytes



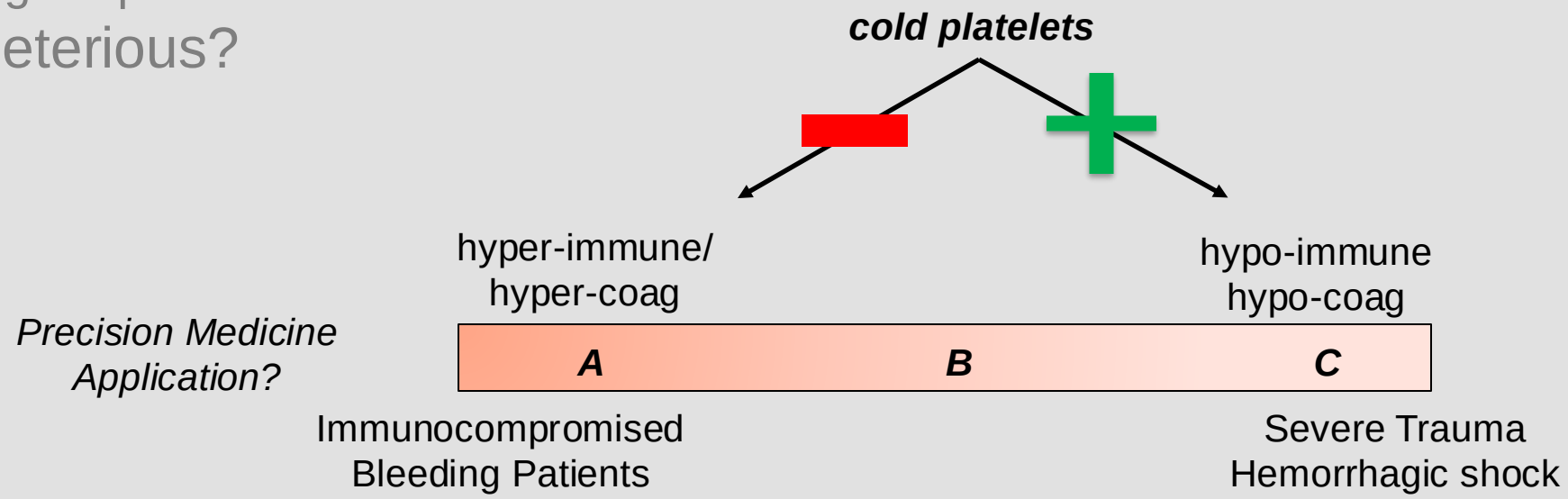
n=9

Immune Summary

- Cold storage for 21 days:
  - D21 metabolic profile was significantly different
  - increased CD62P/P-selectin expression
  - increased myeloid degranulation upon co-culture
  - increased IL-8 production by leukocytes in the presence of LPS
- Long term cold storage modulates platelet:leukocyte interactions *in vitro*

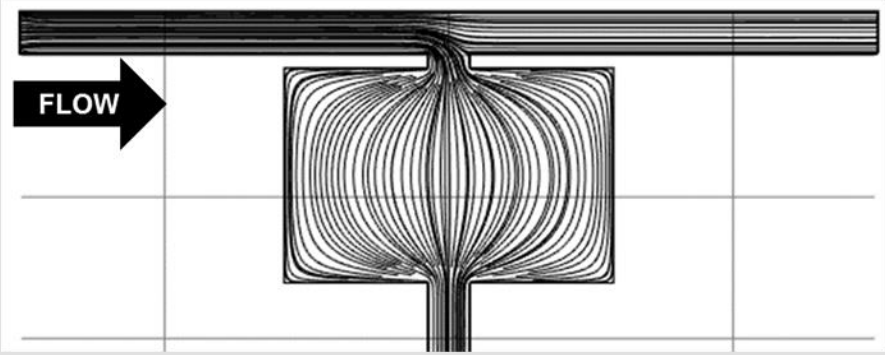
Concomitant Storage Modulation of Coagulation and Immune Function

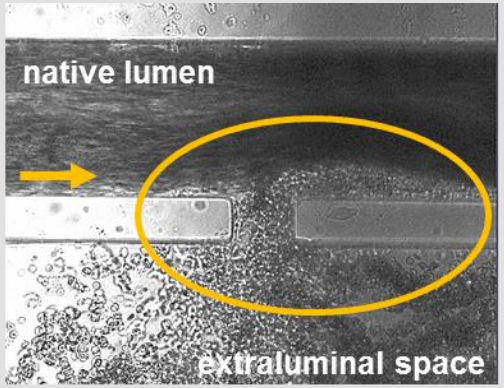
- Platelet hemostatic function is maintained over the duration of cold storage
- Cold storage modulates leukocyte function to exogenous stimuli
- Inflection point between D5 and D10 in both hemostatic and metabolic data
  - Tied to function (MPV vs OT; D21 metabolics and IL8 induction)
  - Non-linear biological phenomena
- Beneficial or deleterious?



Future Directions

- Use microfluidic bleeding chambers to test blood product efficacy
  - Hemorrhage model and transfusion simulation
- Determine mechanisms by which cold stored platelets potentiate immune activation
- Endothelialize microfluidic chambers to start understanding the interplay between hemostatic, immune, and endothelial systems





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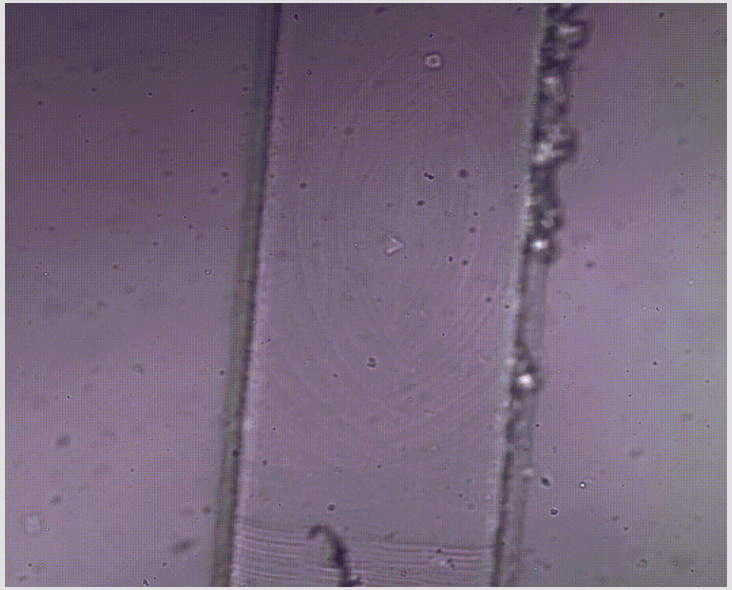


CD41 (Platelets)

Cold Stored Platelets – Plasma vs. PAS

- Purpose:
  - Minimize activation and fibrinogen binding
  - Allow alternative uses for plasma collected
  - Potentially reduces antibody mediated reactions
- Recovery and survival out to 7 days
- Recent work identified citrate promotes lesion formation

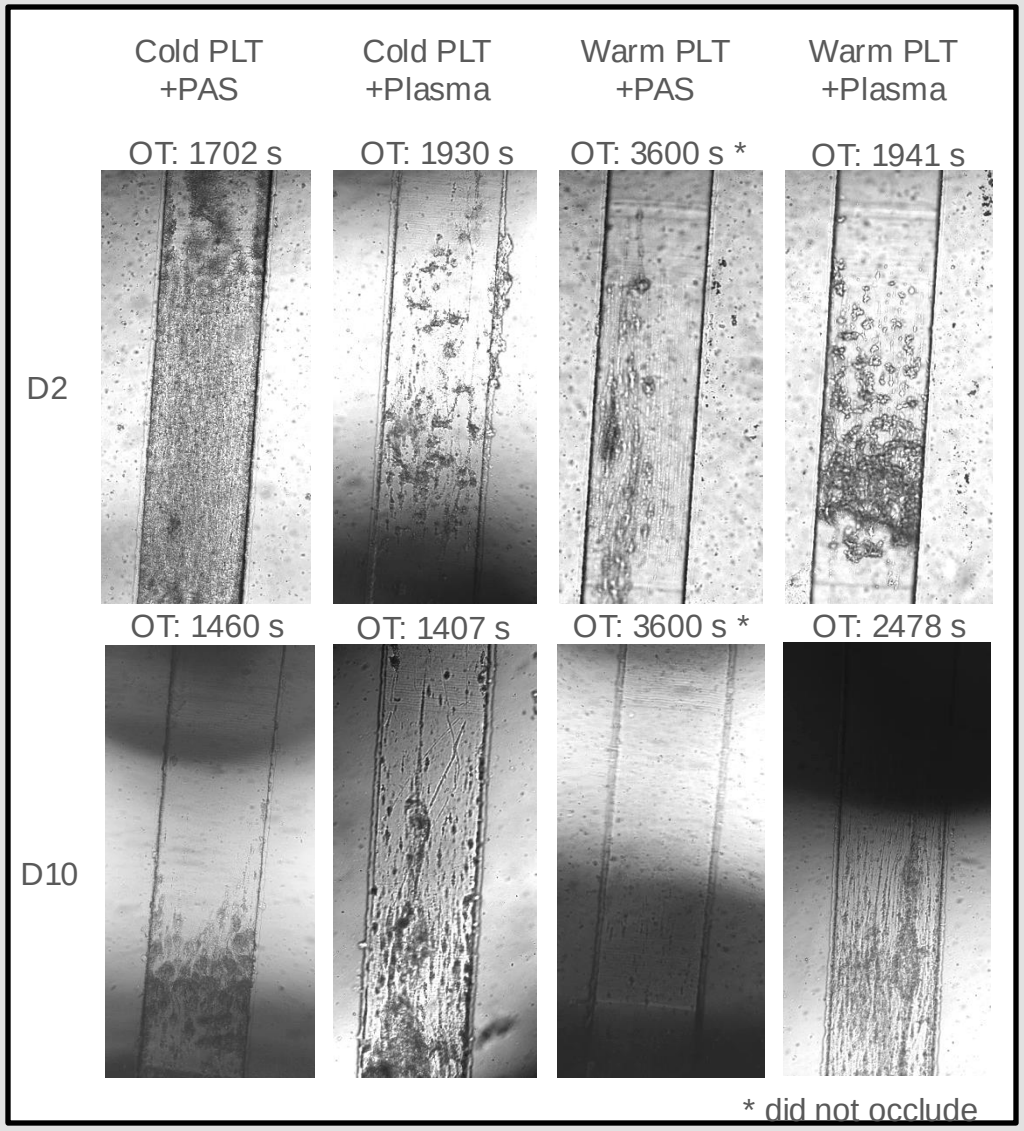
Table of Platelet Additive Solutions

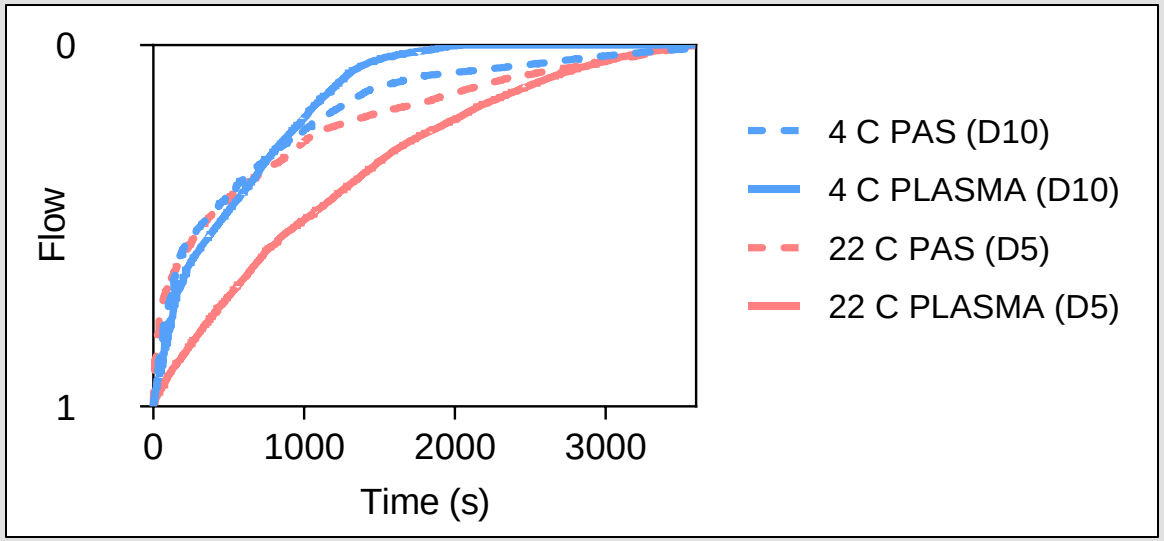
New Name	Citrate	Phosphate	Acetate	Magnesium	Potassium	Gluconate	Glucose	Alternate Names	Previous ISBT 128 Name
PAS	NS	NS	NS	NS	NS	NS	NS		Not named
PAS-A	X	X			X			PAS (1)	Not named
PAS-B	X		X					PAS II, PAS-2, SSP, T-Sol	PASII
PAS-C	X	X	X					PAS III, PAS-3, Intersol	PASIII
PAS-D	X		X	X	X	X		Composol PS	PAS IIIMgK (note, Composol PS should not have been called PASIIIMgK)
PAS-E	X	X	X	X	X			PAS IIIM, SSP+	Not named
PAS-F			X	X	X	X		PlasmaLyte A, Isoplate	Not named
PAS-G	X	X	X	X	X		X		Not named

Source: Ringwald, J., Zimmerman, R., and Eckstein, R: The New Generation of Platelet Additive Solution for Storage at 22°C: Development and Current Experience, Transfusion Medicine Reviews, Vol 20, No 2 (April), 2006: pp 158-164.

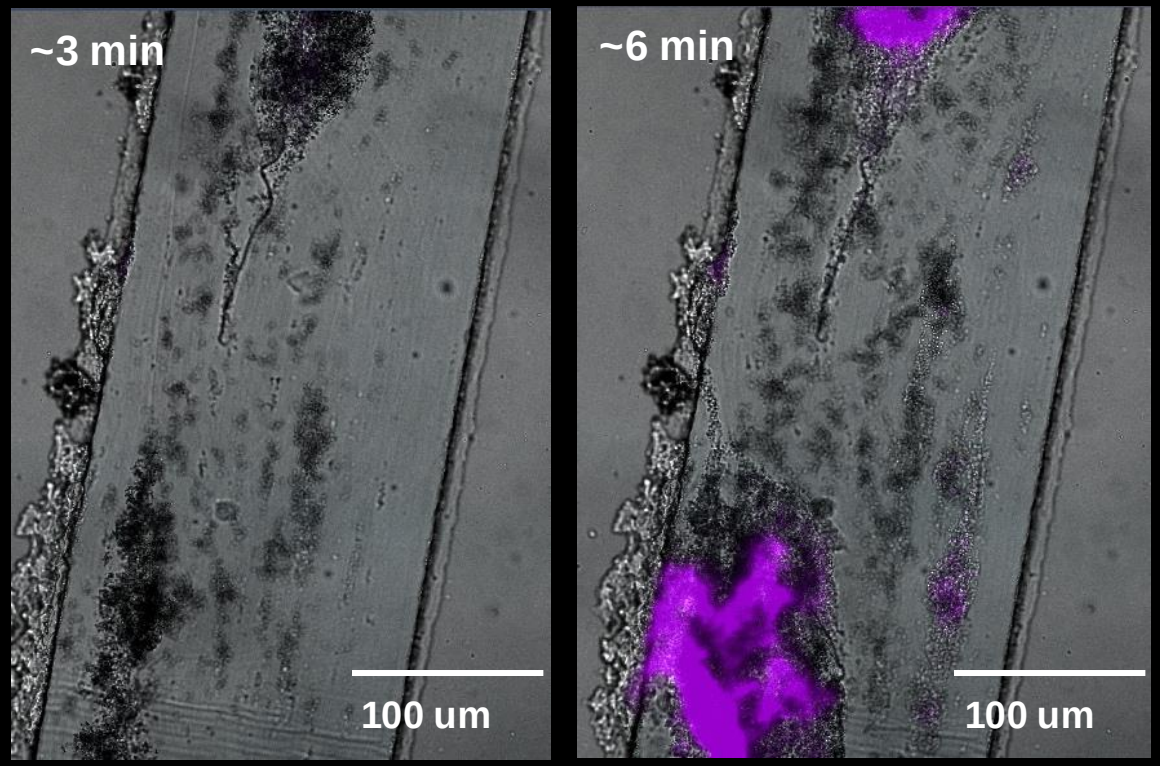
https://www.iccbba.org/tech-library/international-nomenclature/platelet-additive-solution-pas-3

Cold Stored Platelets – Plasma vs. PAS

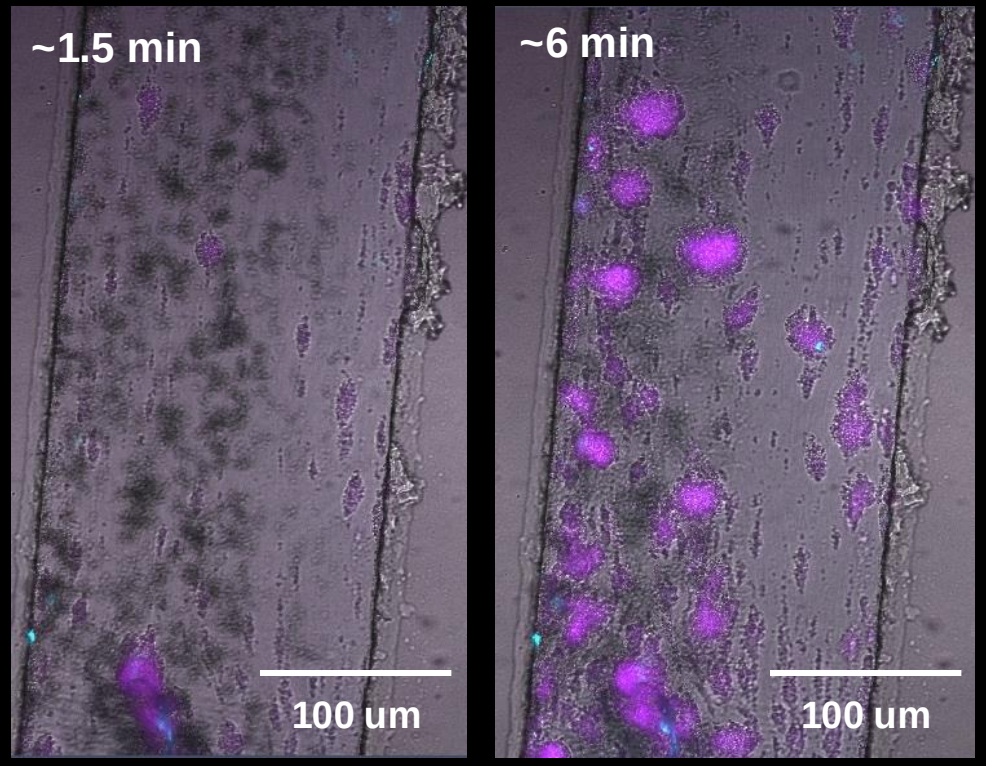




Donor 5, Day 2, 22°°C, Plasma



Donor 4, Day 5, 4°C, PAS



CD41 (Platelets)
vWF (von Willebrand Factor)

SMS

Hemostasis Summary

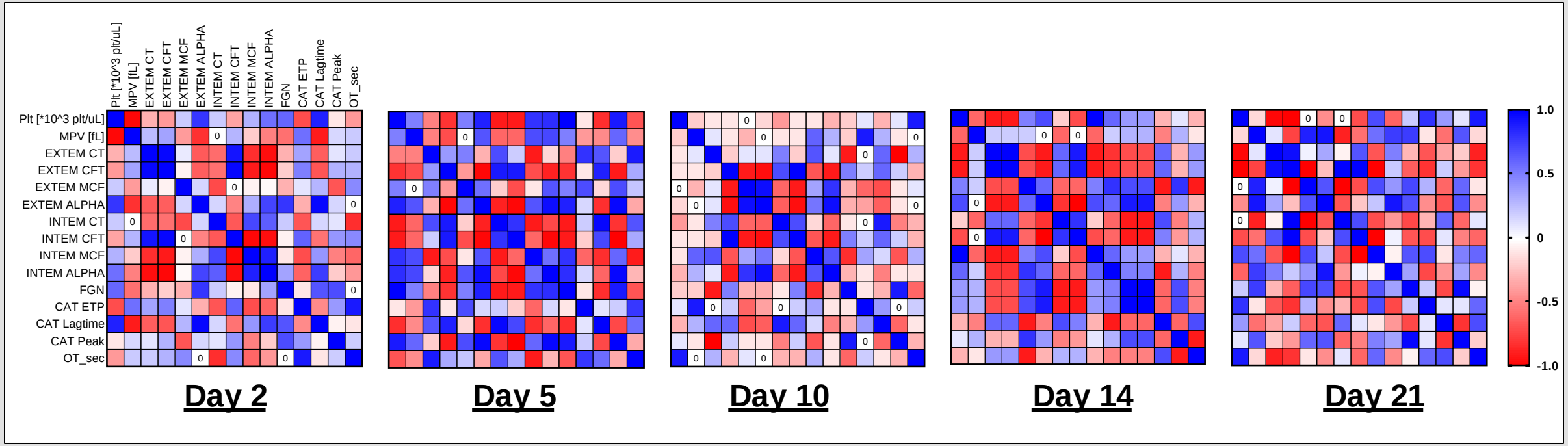
Cold vs Warm

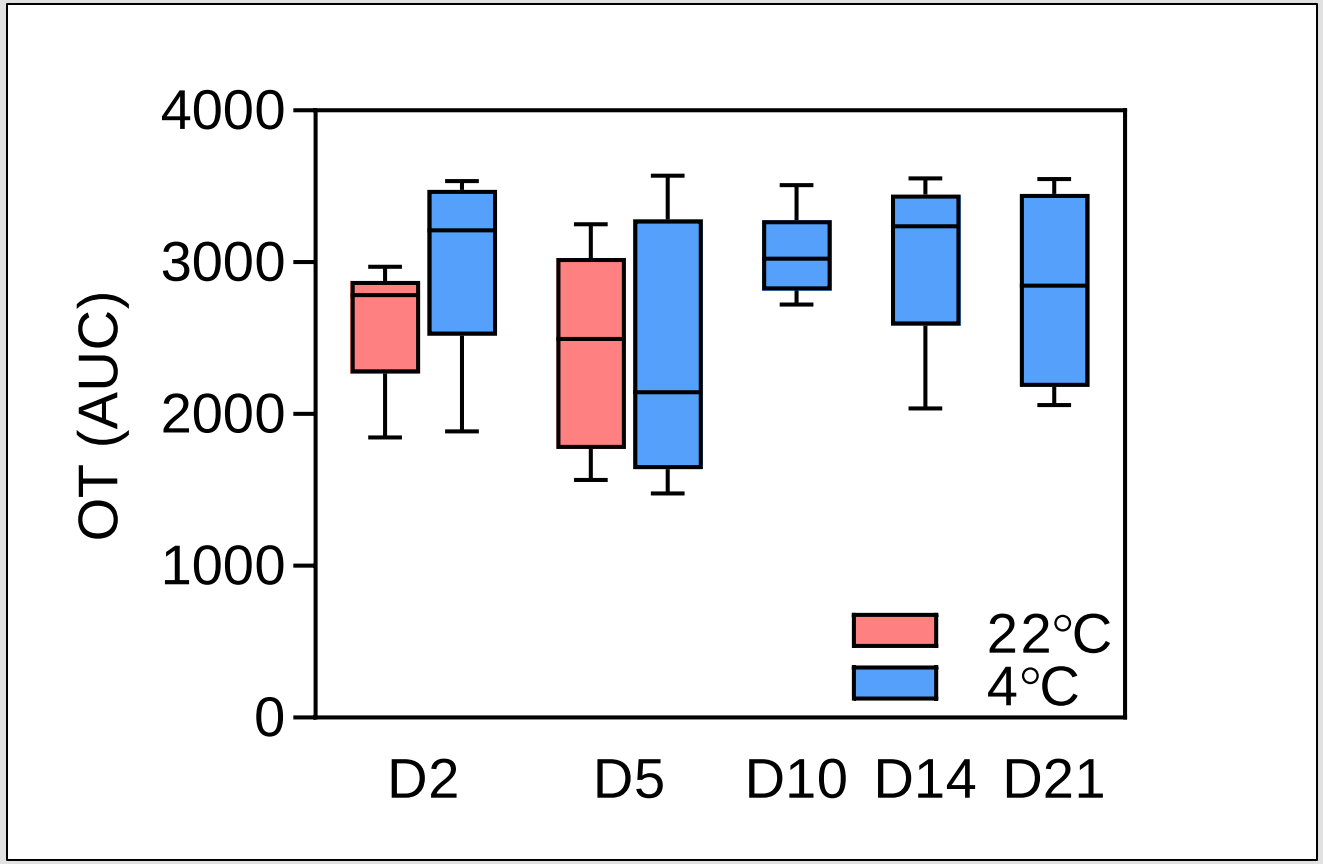
Outcome	Temperature	Time	Donor
EXTEM CT	0.181	0.369	0.200
EXTEM CFT	***0.009***	0.365	0.615
EXTEM MCF	0.714	0.804	0.489
EXTEM ALPHA	0.434	0.349	0.126
EXTEM LI30	***0.004***	0.945	0.172
INTEM CT	***0.004***	***0.011***	0.975
INTEM CFT	***0.001***	0.735	0.213
INTEM MCF	***0.085***	0.558	0.340
INTEM ALPHA	0.402	0.263	0.117
INTEM LI30	0.317	0.317	0.160
ADP AUC	0.111	0.127	0.458
TRAP AUC	0.429	0.653	0.718
COL AUC	0.266	0.623	0.119
Plt Count	0.501	0.468	0.924
MPV	***0.014***	0.251	***0.013***
FGN	0.121	0.089	0.305
OT	0.835	0.374	0.098

Cold Over Time

Outcome	Time	Donor
EXTEM CT	0.295	0.938
EXTEM CFT	***0.002***	***0.009***
EXTEM MCF	***0.000***	0.702
EXTEM ALPHA	0.062	***0.024***
EXTEM LI30		
INTEM CT	0.247	***0.025***
INTEM CFT	***0.000***	0.082
INTEM MCF	***0.000***	0.565
INTEM ALPHA	***0.041***	***0.001***
INTEM LI30		
ADP AUC	***0.013***	0.436
TRAP AUC	***0.005***	0.978
COL AUC	***0.015***	0.208
Plt Count	***0.014***	0.376
MPV	0.215	***0.026***
FGN	0.947	***0.002***
OT	0.306	***0.008***

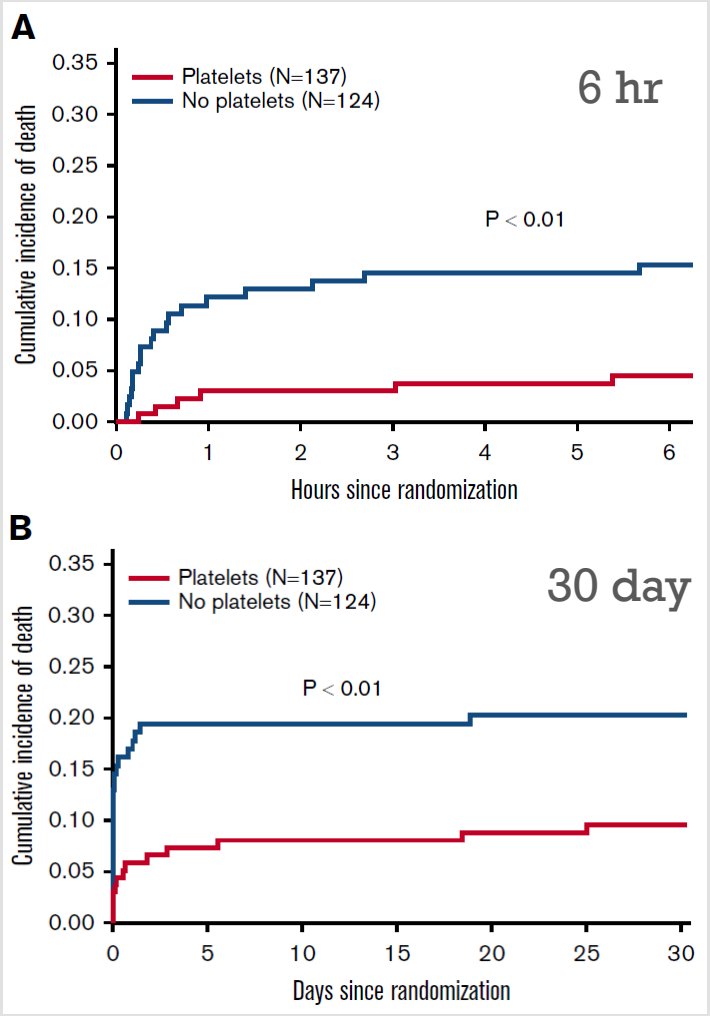
Trends in Cold Storage




4000
3000
2000
1000
0
OT (AUC)
22°C
4°C
D2
D5
D10
D14
D21

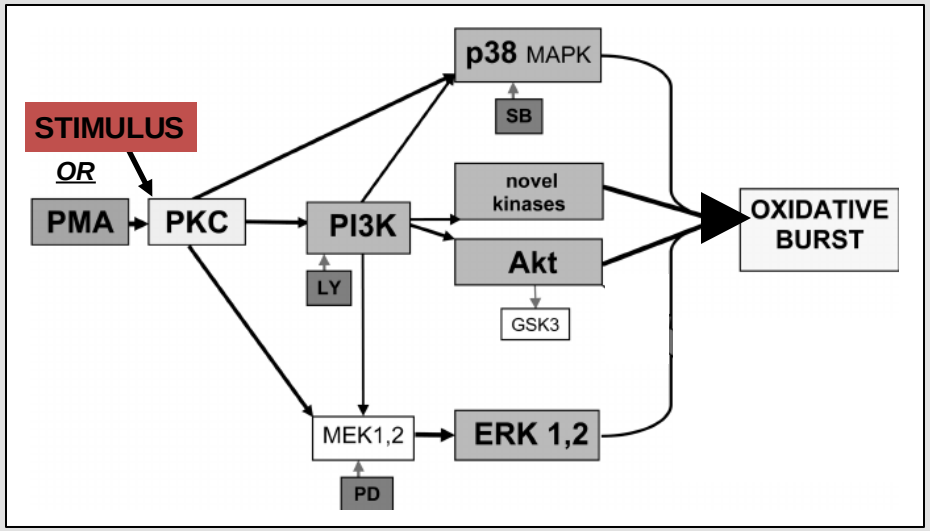
Trauma: Resuscitation & Platelets

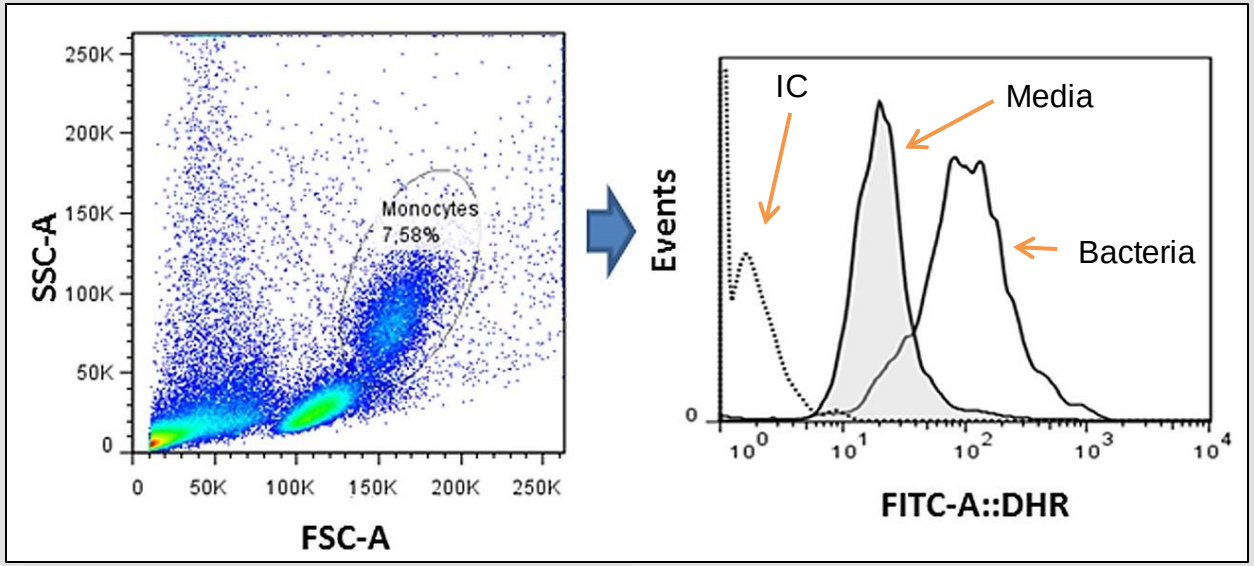
- Damage Control Resuscitation (DCR)
  - Blood product transfusion and use of hemostatic adjuncts to restore hemostasis and improve oxygen delivery
  - Platelets are a crucial component of hemostasis
  - Increased platelet:red blood cell transfusion ratios are associated with decreased mortality (PROMMTT, PROPPR, and ACIT trials)
- Platelet Inventory Issues – Room Temperature Storage
  - Limited shelf life (5 days, US)
  - Required pathogen testing ($$$$ and time)



PMID: 30030268; doi: 10.1182/bloodadvances.2018017699

Respiratory Burst



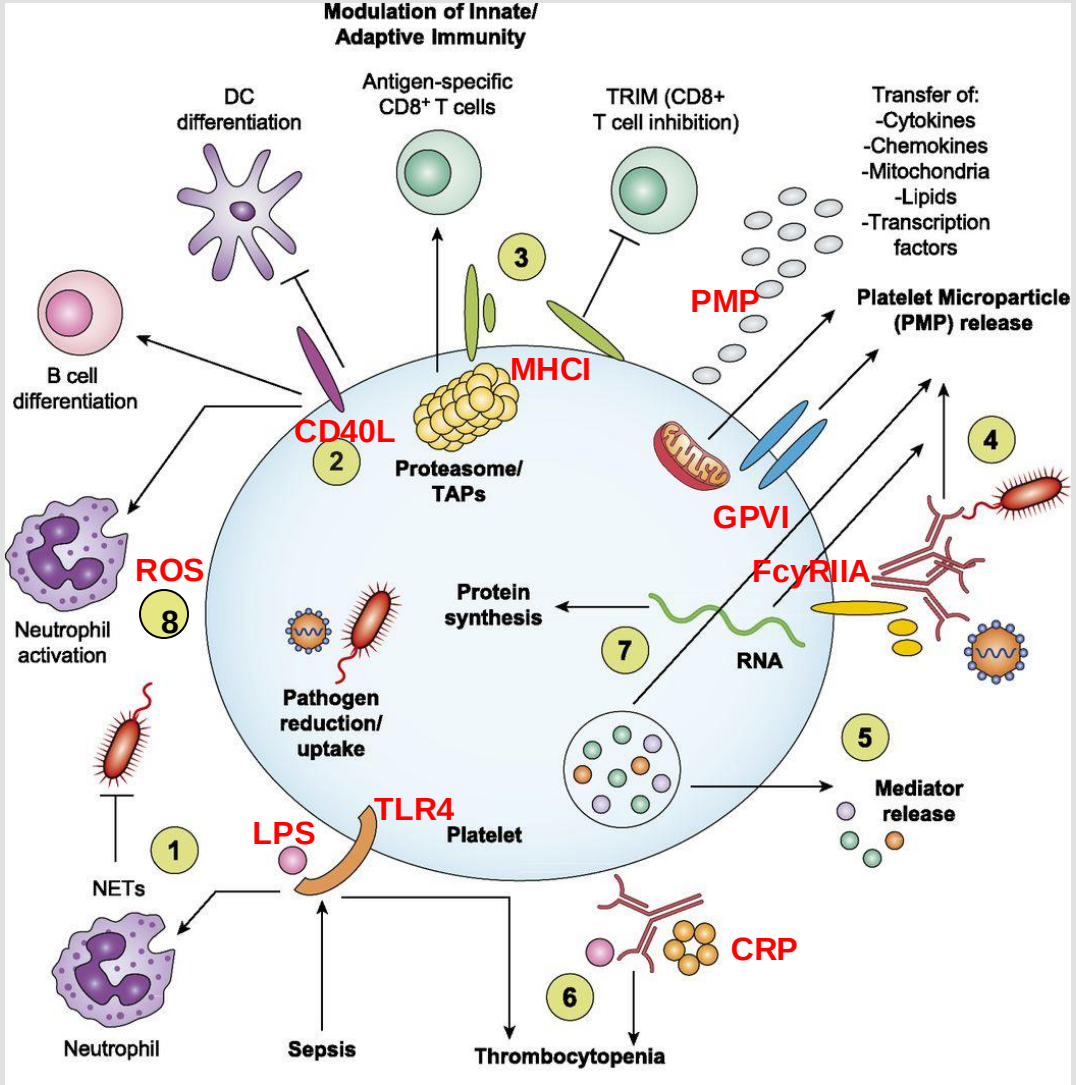


- ROS.

Adapted from doi:10.1093/ndt/gfq731

Adapted from doi:10.1371/journal.pone.0148084

Platelet Immune Function



Assessment

Platelet Metabolic Evaluation = #1-8

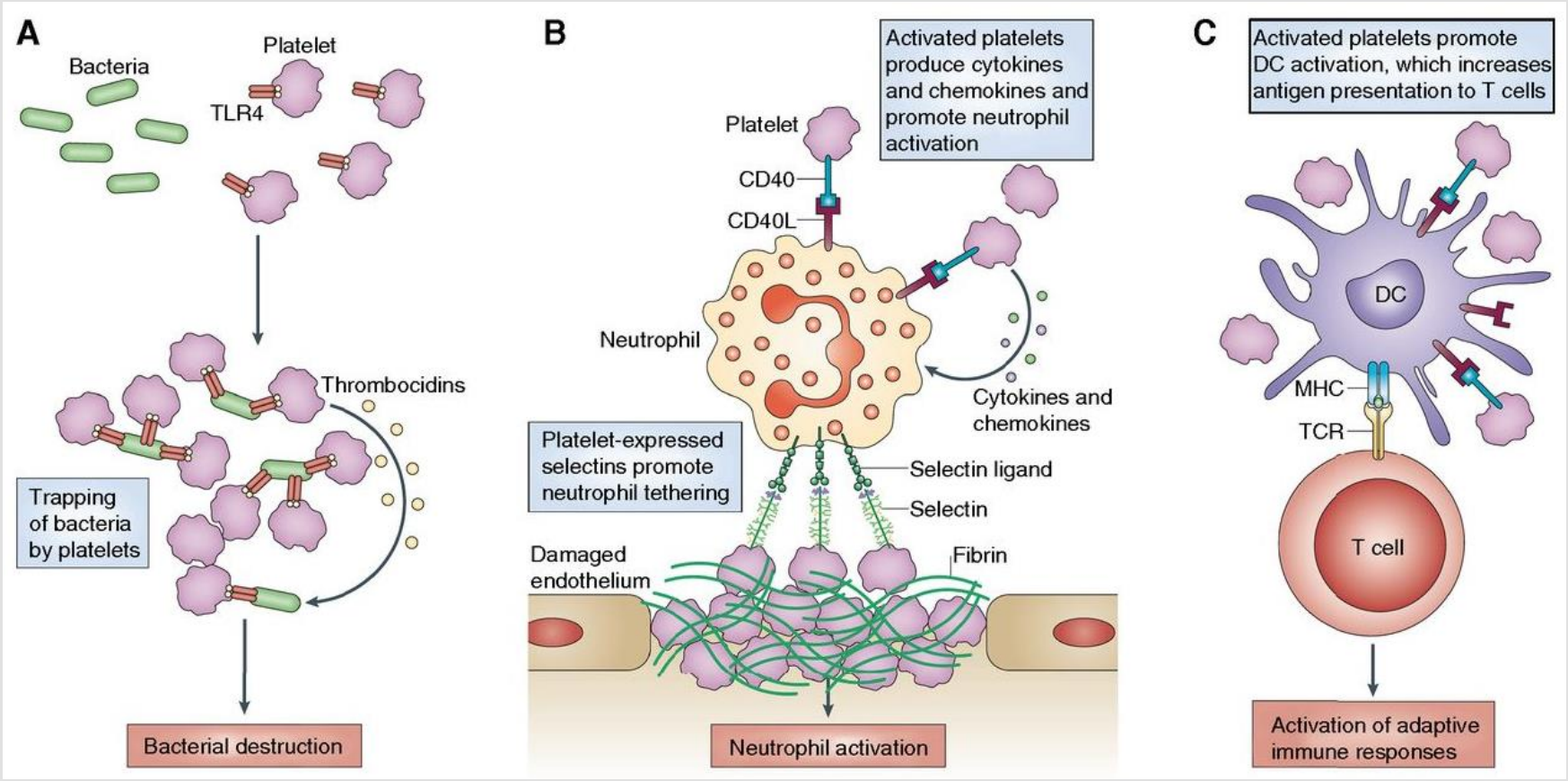
Platelet:Leukocyte co-culture

- Respiratory Burst = #8
- Degranulation = #5

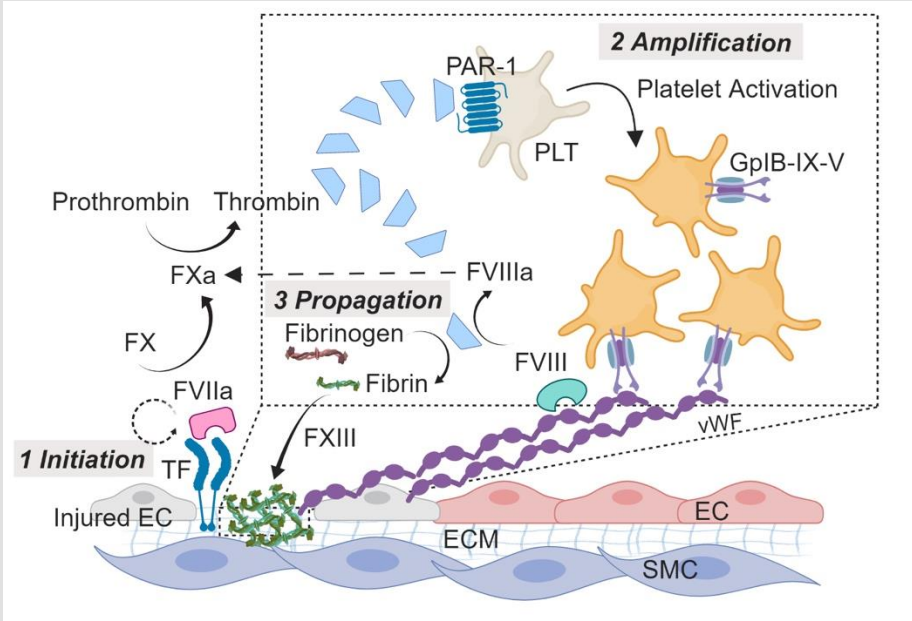
Platelet:Leukocyte co-culture – LPS

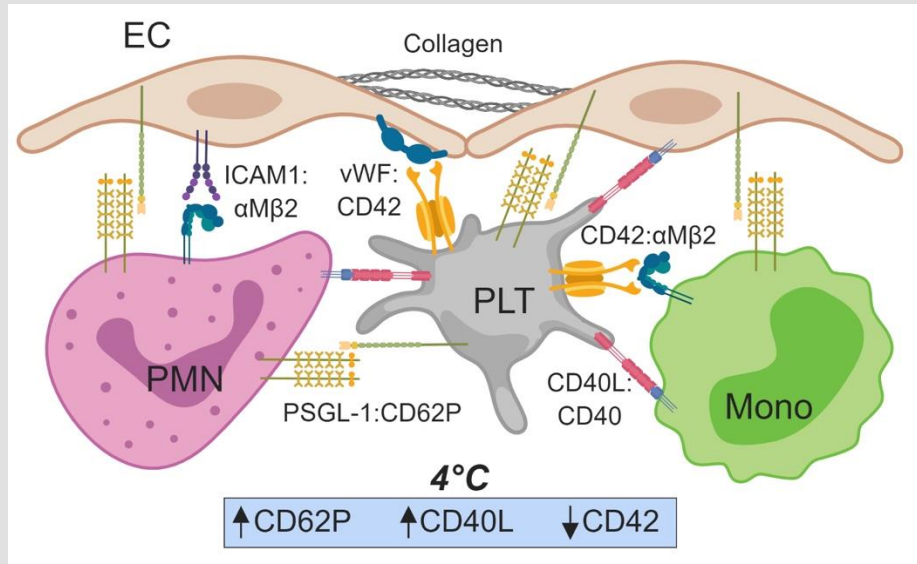
- Cytokine production = #2

Adapted from doi:10.4049/jimmunol.1500259



Adapted from PMID: 27313324; doi.org/10.1182/blood-2016-03-706168


2 Amplification
PAR-1
Platelet Activation
PLT
GpIB-IX-V
Prothrombin
Thrombin
FXa
FVIIIa
3 Propagation
FX
Fibrinogen
Fibrin
FVIII
FVIIa
vWF
FXIII
1 Initiation
TF
Injured EC
EC
ECM
SMC


EC
Collagen
ICAM1:
αMβ2
vWF:
CD42
CD42:αMβ2
PLT
PMN
PSGL-1:CD62P
CD40L:
CD40
Mono
4°C
↑CD62P
↑CD40L
↓CD42