

Platelet response to storage

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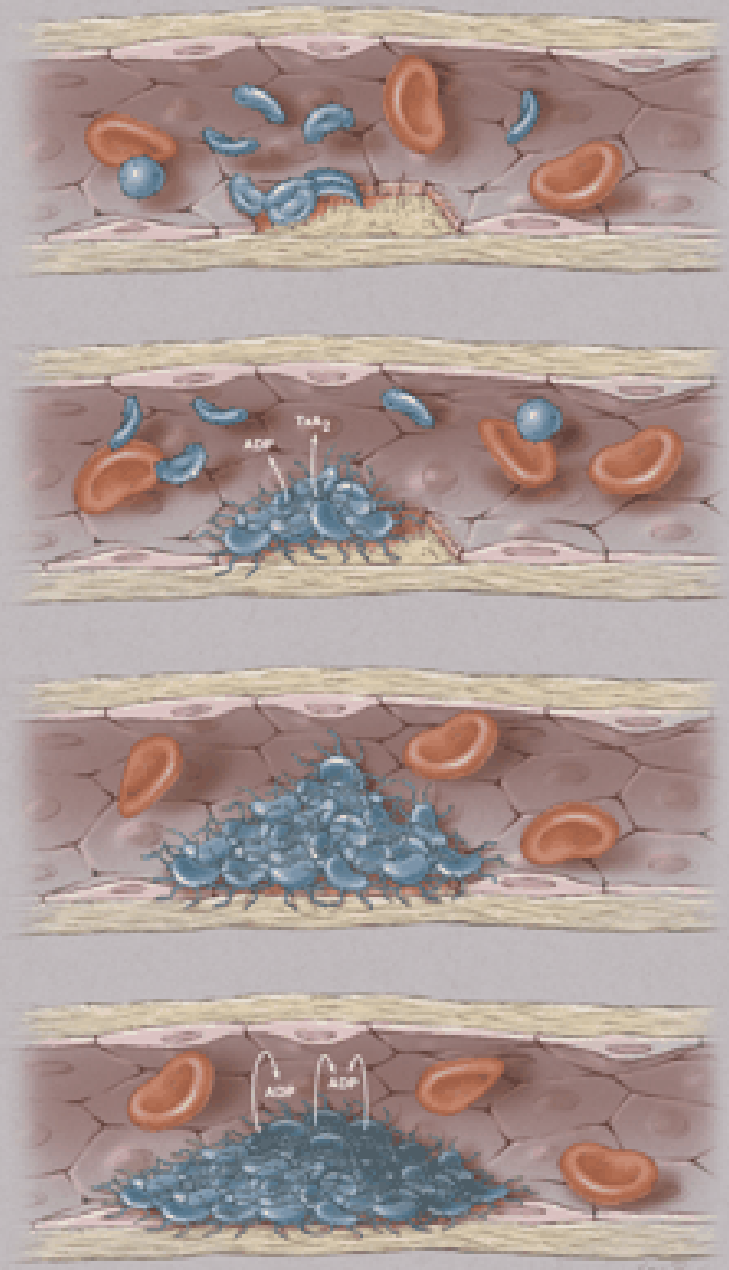
Overview of presentation

- **Platelets – brief review of function and biology**
- **Platelet transfusion products**
 - **Production methods**
 - **Storage and additive solutions**
- **Effects of storage**
- **Assessment**
- **Research**
- **Burning questions**

Platelets

> **Primary function:**

> **to prevent bleeding**



Breach in blood vessel wall



Platelet adhesion



**Thrombus formation
“platelet plug”**

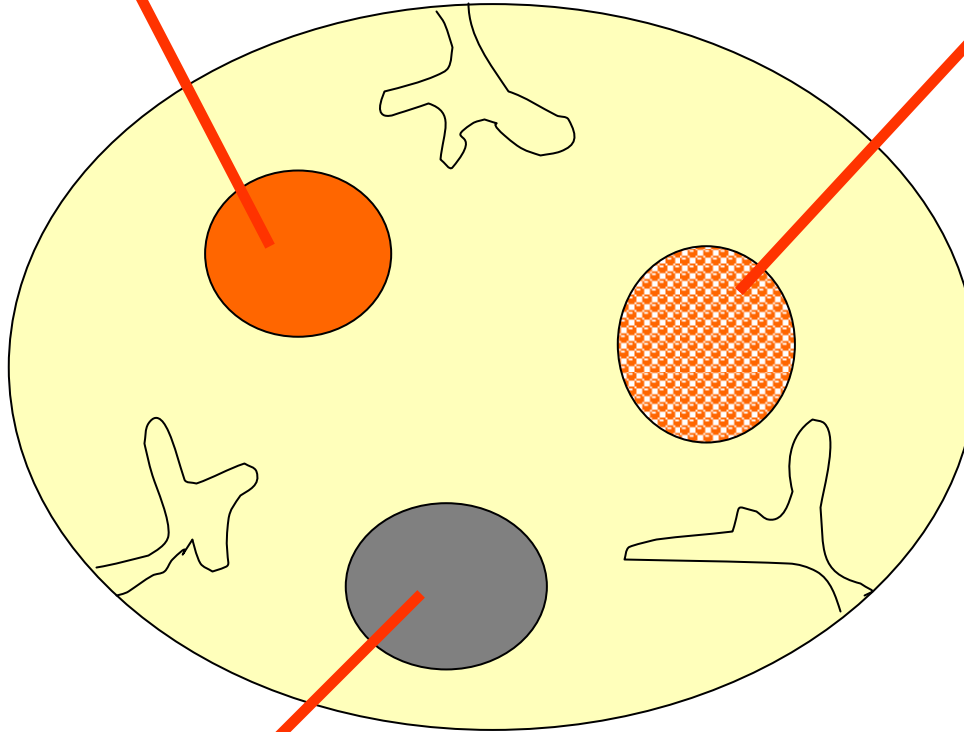
Dense granule

(early marker of activation, released prior to α granule release):

Serotonin

ADP / ATP

Calcium



α -granule

Platelet basic protein

PAF4

RANTES

PDGF

TGF- β

CD62P

vWF

Factor V, XIII

Fibrinogen

Lysosome

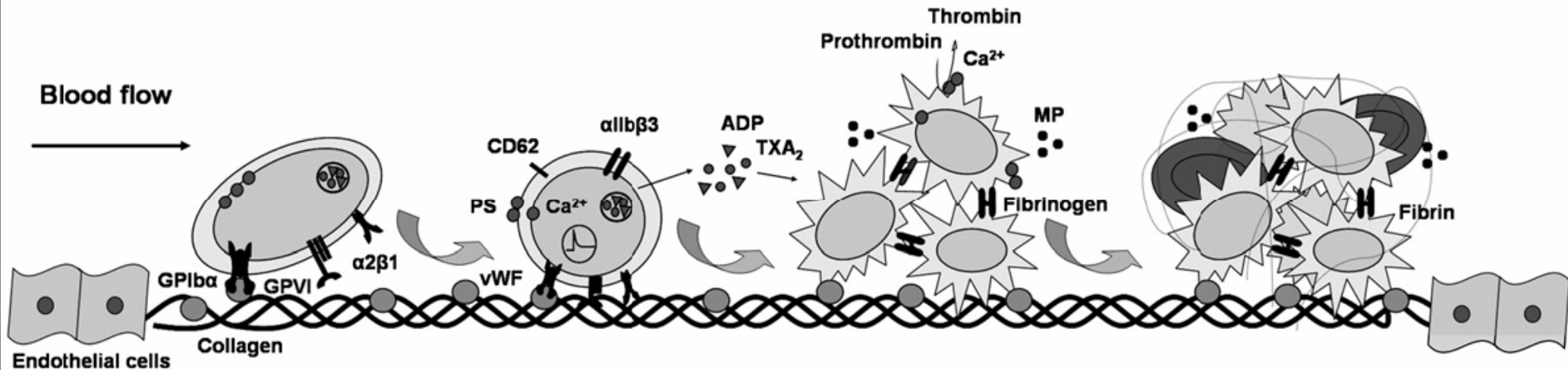
CD63

hydrolytic enzymes

Platelet adhesion

Thrombus formation

Clot formation



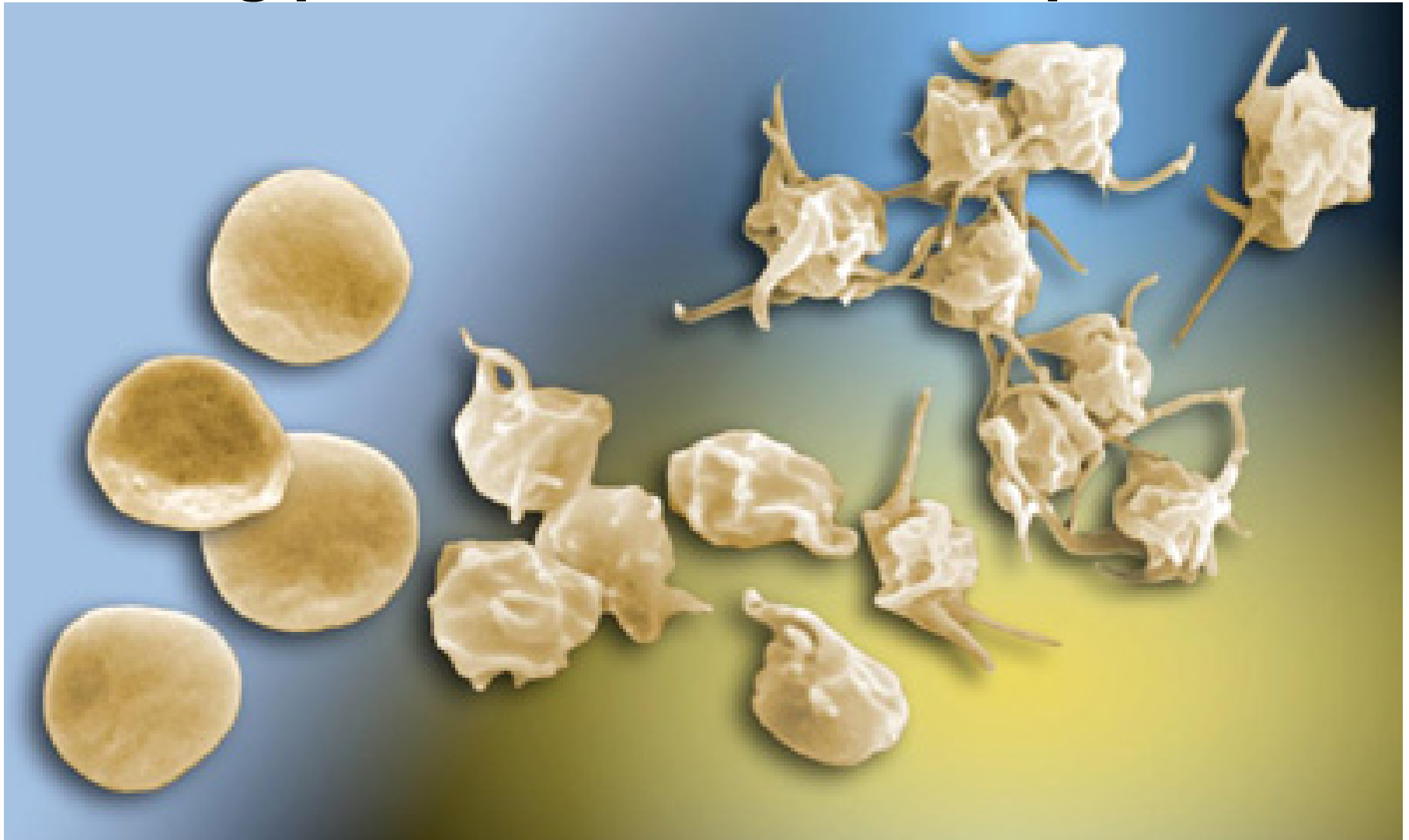
Translocation
Firm adhesion
Shape change

Secretion
Inside-out signalling
Aggregation
Outside-in signalling

Procoagulant activity
Vesicle formation
Clot retraction

Resting platelets

Activated platelets



Circulating lifespan: ~10 days

Platelet concentrates (PC) for transfusion

Production methods

Whole blood-derived PC



- > pools of 4 – 5 donors
- > platelet rich plasma (PRP)
 - > plasma suspension
- > pooled buffy coat (BC)
 - > plasma or additive solution



Plateletpheresis

- > single donor
- > plasma suspension

Platelet storage

- **Gas permeable bags**
 - **allows aerobic metabolism, preserves neutral pH by minimising lactate production**
- **20 - 24°C storage**
 - **slower aging of platelets compared to 37°C**
- **Gentle agitation [reciprocal shaker]**
- **5-day shelf-life**

Additional treatments

- > Irradiation – to prevent TA-GvHD
- > Pathogen inactivation

Quality criteria

- > Platelet count: $> 2 \times 10^{11}$ /unit
- > pH 6.4 – 7.4
- > Leucocyte depletion (pre-storage filtration)
 - < 1 million WBC/unit
- > Bacterial screening

Platelet additive solutions

- > **Gold standard - Plasma**
- > **Synthetic additive solutions**
- > **Advantages**
 - > reduced amount of plasma transfused
→ reduced adverse reactions
 - > more plasma for fractionated plasma products
 - > pathogen inactivation by photochemical treatment
- > **Disadvantages**
 - > platelet function not preserved as well as plasma-suspended platelets

Platelet Additive Solutions (PAS)

currently available formulations (in mM)

	PAS-II [T-Sol] (Baxter)	PAS-III [InterSol] (Baxter)	PAS-IIIM [SSP+] (MacoPharma)	Composol (Fresenius)
NaCl	116	77	69	90
Na₃ citrate	10	10	10	11
Na acetate	30	30	30	27
Na phosphate	-	26	26	-
Na gluconate	-	-	-	23
KCl	-	-	5	5
MgCl₂	-	-	1.5	1.5

New experimental formulations under development

Effects of storage on platelets

	Platelet response	Effect of platelet storage
Adhesion	Translocation	Cleavage of GPIb α , V; clustering of GPIb-V-IX → platelet clearance
	Firm adhesion	↓ adhesion under flow
	Shape change	Loss of discoid morphology; ↑ [Ca ²⁺] _i → cytoskeletal reorganisation
Thrombus formation	Secretion	↓ Agonist-induced Ca ²⁺ and secretion response
	Inside-out signalling	Activation of integrin / receptor molecules → ↓ agonist-induced integrin activation
	Aggregation	↓ Agonist-induced aggregation
	Outside-in signalling	↑ Proteolysis → receptor cleavage
Clot formation	Procoagulant	↑ PS exposure; → ↑ clearance
	Vesicle formation	Accumulation of procoagulant vesicles
	Clot retraction	? Retained

Factors that influence PC quality

- **Production method**
 - **PRP**
 - **Buffy coat**
 - **Plateletpheresis**
- **Suspension medium – plasma vs PAS**
- **Storage conditions and handling**

- **Donor variability**
 - **Inherent genetic platelet responsiveness**
 - **Co-existing (sub)-clinical conditions**

Methods to assess platelet concentrates

- > ***in vivo* survival – 1 hr & 24 hr increments**
 - > **24 hr platelet survival (radiolabel method)**
- > ***in vivo* function - recipient responses**
 - > **Bleeding stopped?**
- > ***in vitro* assessment of platelet concentrates**

Methods for *in vitro* assessment of PCs

Test	Correlation with recovery or survival reported?	Comments	Suitable as routine test for PC quality?	
Morphology	Kunicki score; ESC; swirling	Yes	Labor intensive and/or subjective	No
Aggregation / Adhesion	Aggregometry; PFA-100; Impact-R	No	Labour intensive and/or not easily applied to PC	No
Integrin expression	CD62P; GPIb	(Yes)	Labour intensive – flow cytometry	No
Procoagulant activity	PS exposure; vesicle generation	No	Labour intensive – flow cytometry	No
Metabolism	pH; lactate; HSR; ATP	Yes (extremes)	pH simple; others more demanding	Yes (pH)
Clot formation / retraction	Thromboelastograph	No	Depends on coagulation as well platelet function	No

What else do we need to know?

Accumulation of platelet-derived cytokines in the supernatant of PCs during storage

Accumulation of platelet-derived cytokines in the supernatant of BC-PCs during storage

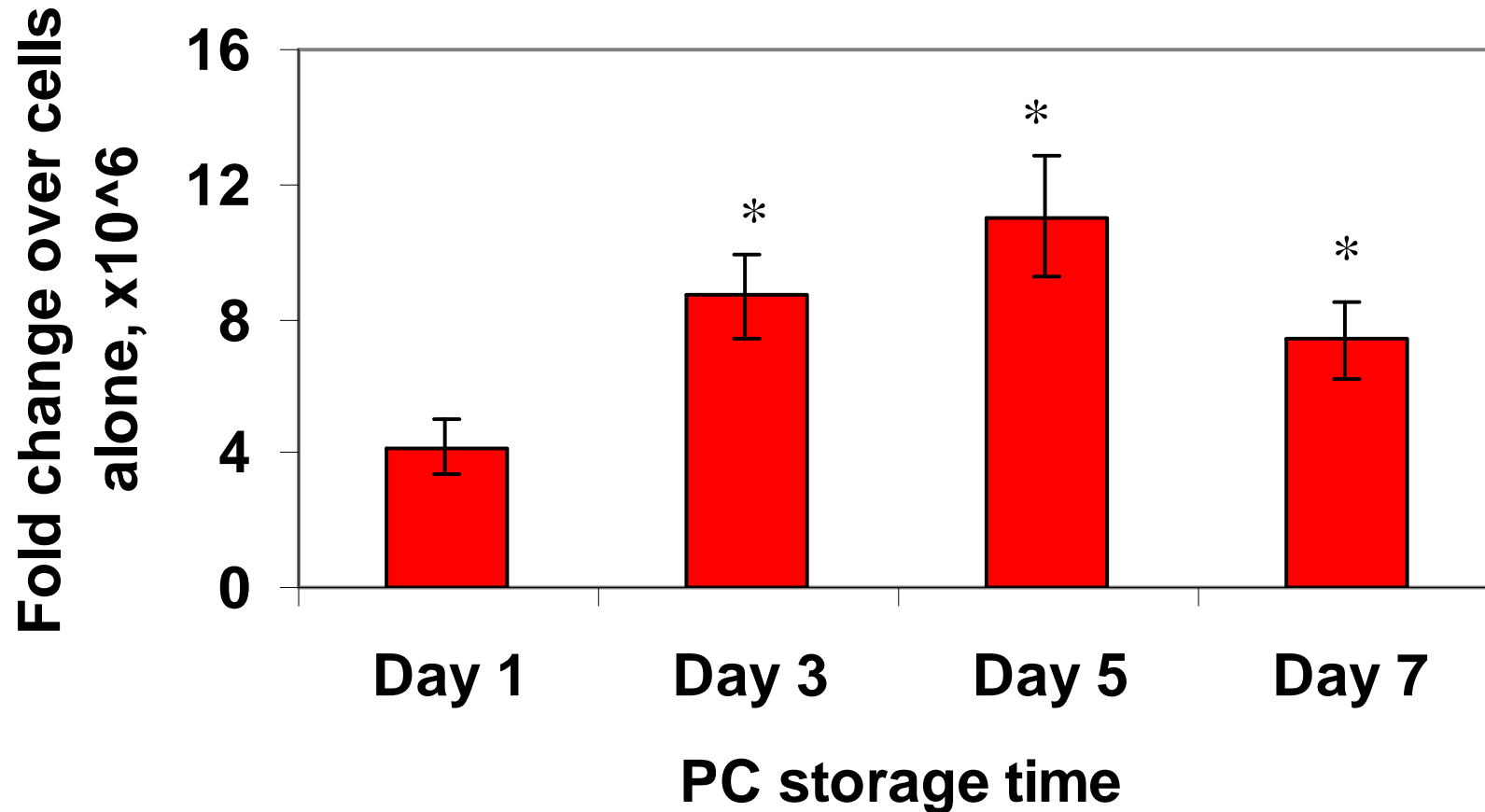
	Cytokine concentration (ng/ml)		
	Day 1	Day 7	Plasma
RANTES	124	404*	1
PBP/NAP2	3,200	10,600	200
sCD40L	2.7	8.8*	0.67
BDNF	20	49	0.16
PDGF-BB	2.2	5.8	0.1
EGF	0.15	0.82	0.02
VEGF	0.09	0.13	0.002

n > 10

* Reported biologically significant levels

**PC supernatant stimulates
allogeneic neutrophils to release
immunosuppressive cytokine, IL-1ra**

PC supernatant stimulates allogeneic neutrophils to release immunosuppressive cytokine, IL-1ra



induced release of 0.3-1.9 ng/mL IL-1ra compared to PMNs alone

Other areas of research

> Cold storage of platelets

- > galactosylation to block phagocytic receptor
- > hopes dashed by recent *in vivo* studies
 - > Wandall H et al Blood 2008;111:3249-56

> Lyophilised platelets

- > ? reduced haemostatic function
- > ? useful for wound healing

> Platelet microparticles

- > ? haemostatic potential

> **Platelet lifespan**

> **can the internal clock be manipulated to increase platelet lifespan?**

> **Mason K et al Cell 2007;128(6):1173-86**

> **Understanding the genetic factors that determine inherent variability in platelet responsiveness**

> **will we eventually screen platelet donors based on inherent responsiveness?**

> **Garner S et al Transfusion 2008;48:673-80**

Burning questions!

- > **Why do we transfuse platelets?**
 - > **Prophylactic vs major bleeding**

- > **What function do we need them to perform?**
 - > **Prophylactic – need unactivated resting platelets**
 - > **Major bleeding – perhaps activated platelets might be beneficial ??**

- > **What do we want *in vitro* tests to measure?**
 - **recovery, survival or haemostatic responsiveness?**

- > **Can we store PCs for longer?**
 - > **Bacterial contamination concern**
 - > **Cold storage**
 - > **Lifespan studies**

- > **What is the immunomodulatory potential of PCs?**

- > **Are there other novel clinical uses for PCs?**

Recommended references

Platelet function and assessment

- > Cardigan R et al Vox Sang 2005;88:153-63
- > Cauwenberghs S et al Transfusion Med Rev 2007;21(4):287-94
- > Maurer-Spurej E, Chipperfield K Transfusion Med Rev 2007;21(4):295-306
- > Furie B & Furie BC J Thromb Haemost 2007;5(suppl 1):12-7

Platelet adhesion receptors

- > Berndt MC et al J Thromb Haemost 2007;5(suppl 1):212-9

Platelet production methods

- > Vassallo R, Murphy S Curr Opin Hematol 2006;13:323-30

Platelet additive solutions

- > Gulliksson H. Immunohematology 2007;23(1):14-9

Recommended references (cont)

Donor related inherent platelet responsiveness

- > Garner SF et al Transfusion 2008;48(4):673-80

Cold storage of platelets / galactosylation

- > Wandall HH et al Blood 2008;111(6):3249-56
- > Lozano M Blood 2008;111(6):2951 (editorial)
- > Hornsey VS et al Vox Sang 2008 (early-on-line, April 2008)

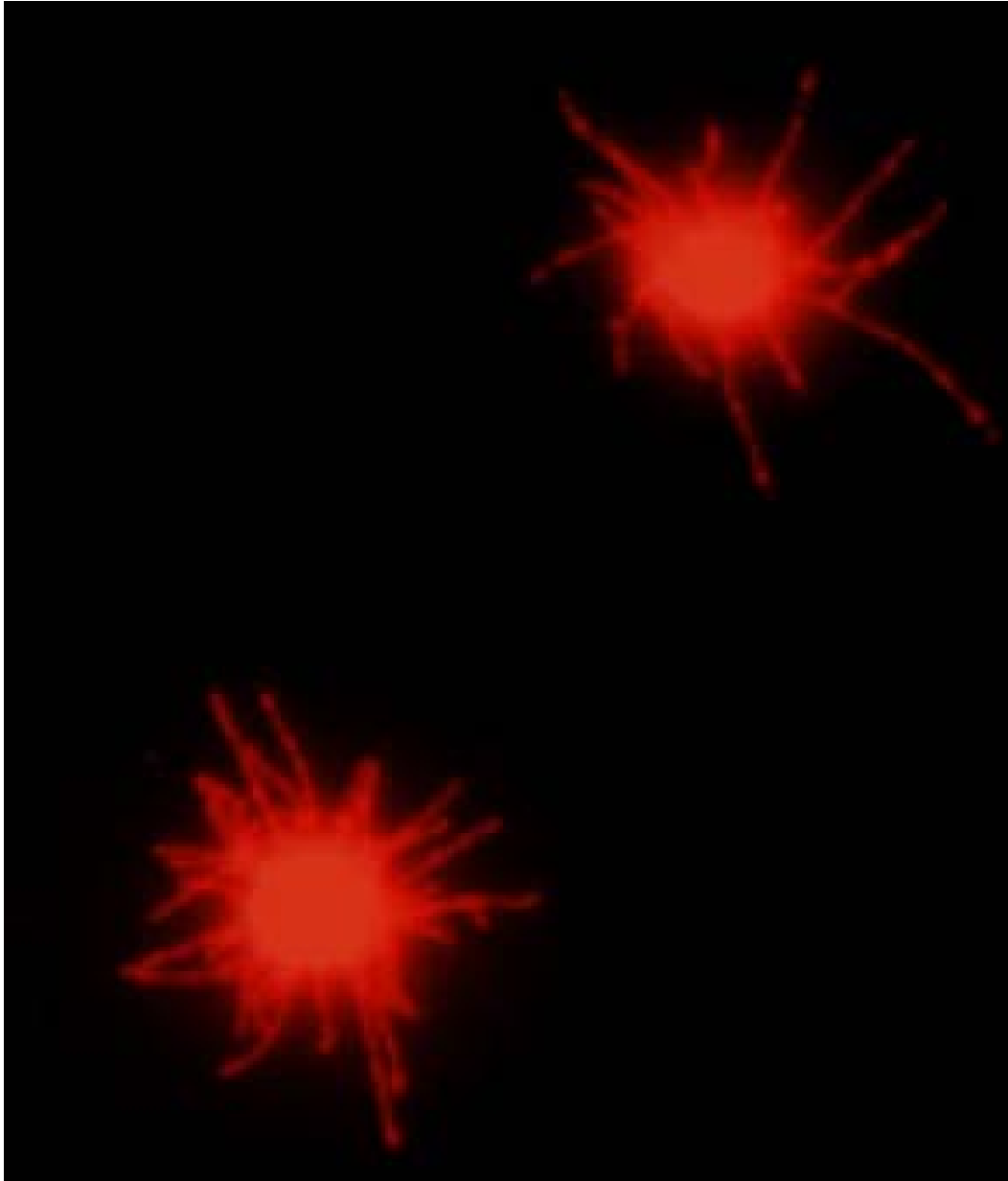
Lyophilised platelets

Wound healing

- > Sum R et al Transfusion 2007;47(4):672-9.

Haemostatic function

- > Fischer TH et al Transfusion 2006;46:1943-50



Fluorescent, PKH26-labelled activated platelets

- photo by Dr Margaret Veale