Platelet Storage: Putting the biology to the test

Nash J[1], Saunders C[2], Davies A[1] and James P[1]

[1]*Cardiff Metropolitan University*

[2]*Welsh Blood Service, NHS*

British Blood Transfusion Society | #BBTS2019

Platelet Storage – 7-day life

- Red Book Guidelines –
  - 22°C±2°C
  - Gentle Agitation
  - Shelf Life of 7 Days (With Bacterial Monitoring)
  - Gas Permeable Bags

Guidelines for the Blood Transfusion Services in the UK, 8th Edition

Platelet Storage Lesion

- Biochemical and mechanical changes that occurs over platelet concentrate storage causing a deteriorated quality over time[1]
- Impeding the lesion will
  - A) Possibly give a longer storage time

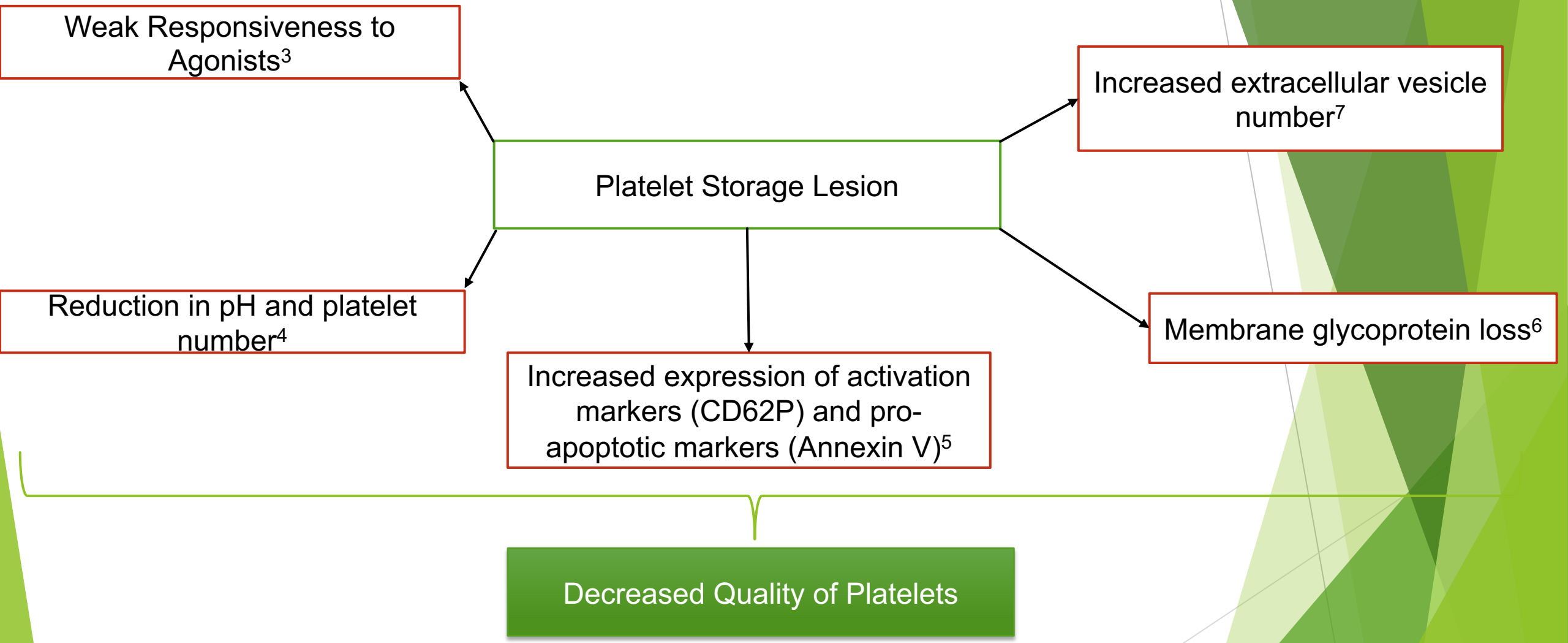
  and/or

  - B) Give a better quality product at end of storage

***In-Vitro* tests**
Platelet Structure
Swirling Phenomena
Morphology by microscopy
Functional Tests
Aggregation studies
Hypertonic shock response
Extent of change
Metabolic Status
pH
Glucose and lactate levels
Activation
CD62P surface and supernatant levels
Annexin V binding

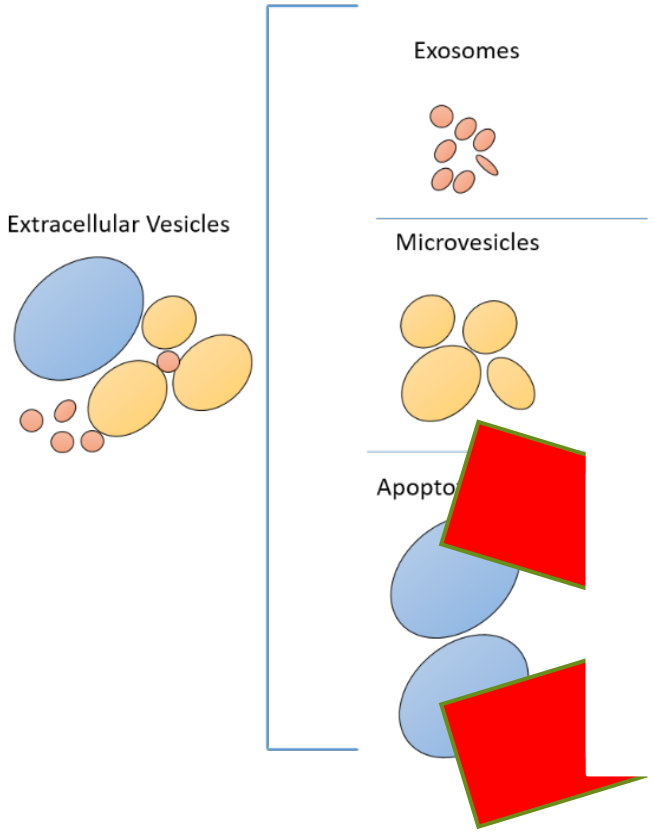
In-Vitro tests of Platelet Quality. *Adapted from Snyder et al 2007*[2]

Consequences



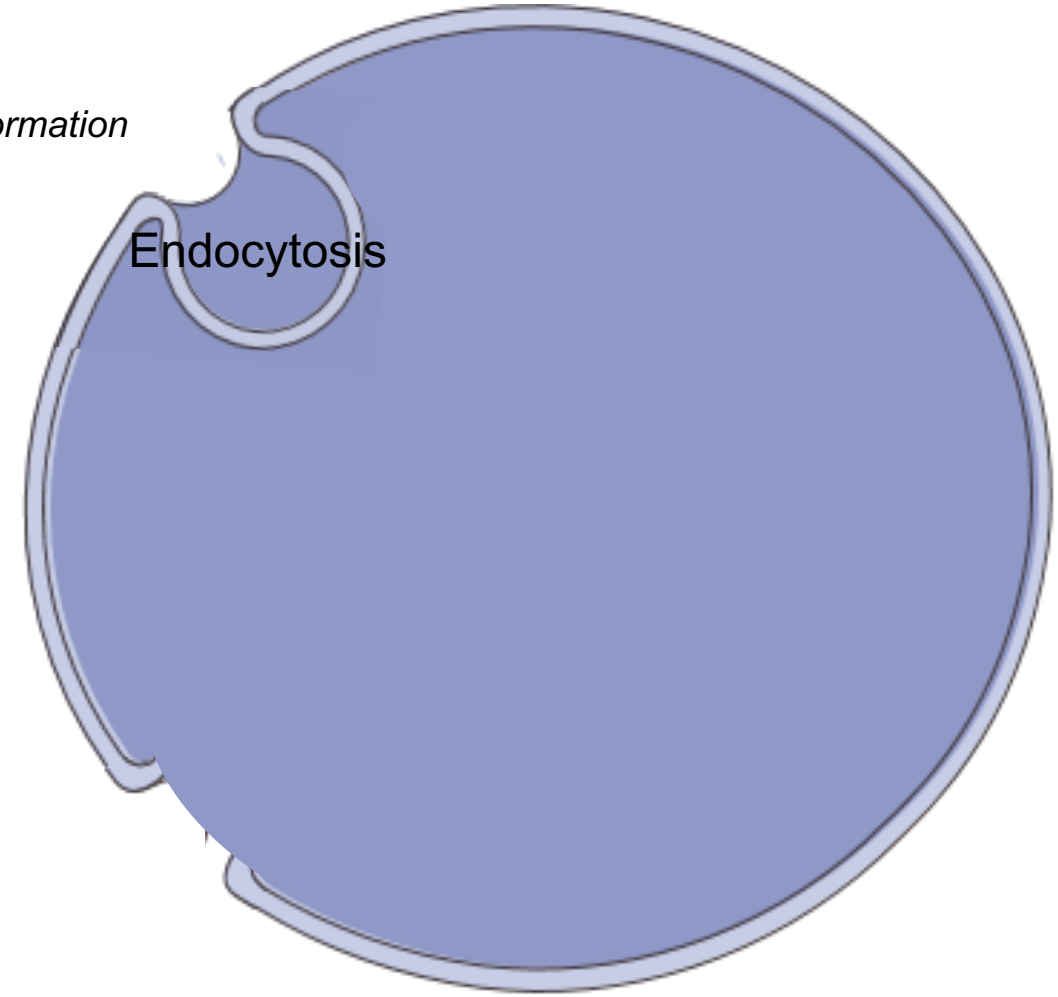
Extracellular Vesicles

Name	Size	Origin	Markers
Exosomes			
Microvesicles			
Apopto			



Exosomes

Endosome formation



Intraluminal vesicles (ILVs) formation

Release of the exosome is facilitated by the fusion of MVBs with the plasma membrane

Microparticles

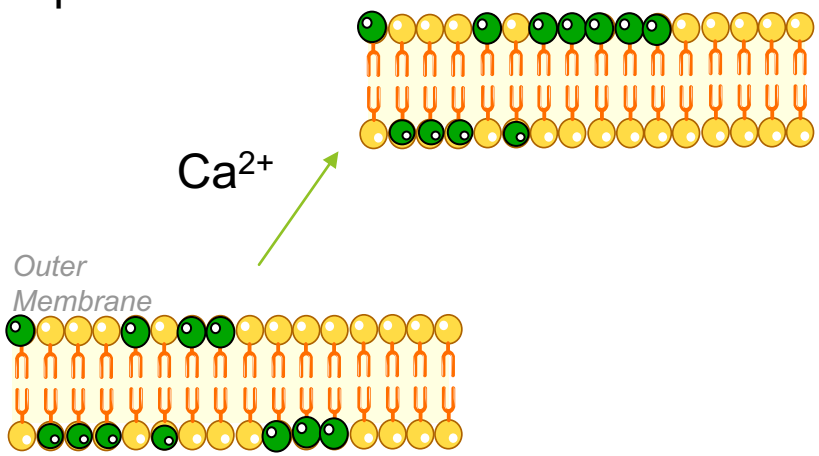
Phosphatidylserine

Other Phospholipids

Flippase internalises PS.

Floppase externalises, both using ADP.

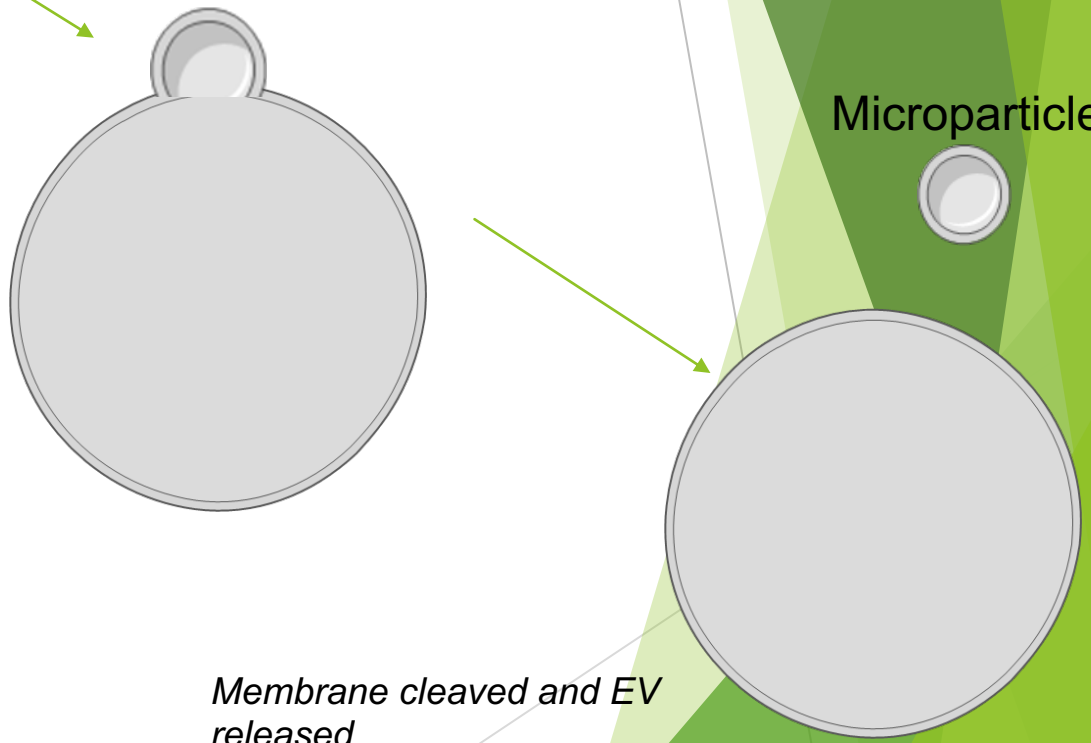
Scramblase uses bi-directional ADP independent translocation.



Resting state, Flippases rate of work is higher

Upon cellular activation, Calcium inhibits flippase, leading to floppase and scramblase disrupting asymmetry.

PS externalisation along with disruption to the actin cytoskeleton.



Membrane cleaved and EV released

Platelet EVs (PEVs)

- Formation of PEVs relies on a rise in intracellular calcium and can be induced by platelet activation or cell death (Microvesicle Pathway).
- 104PEVs/ul in blood[12]
- 50-100 times more pro-coagulant than platelets[12]

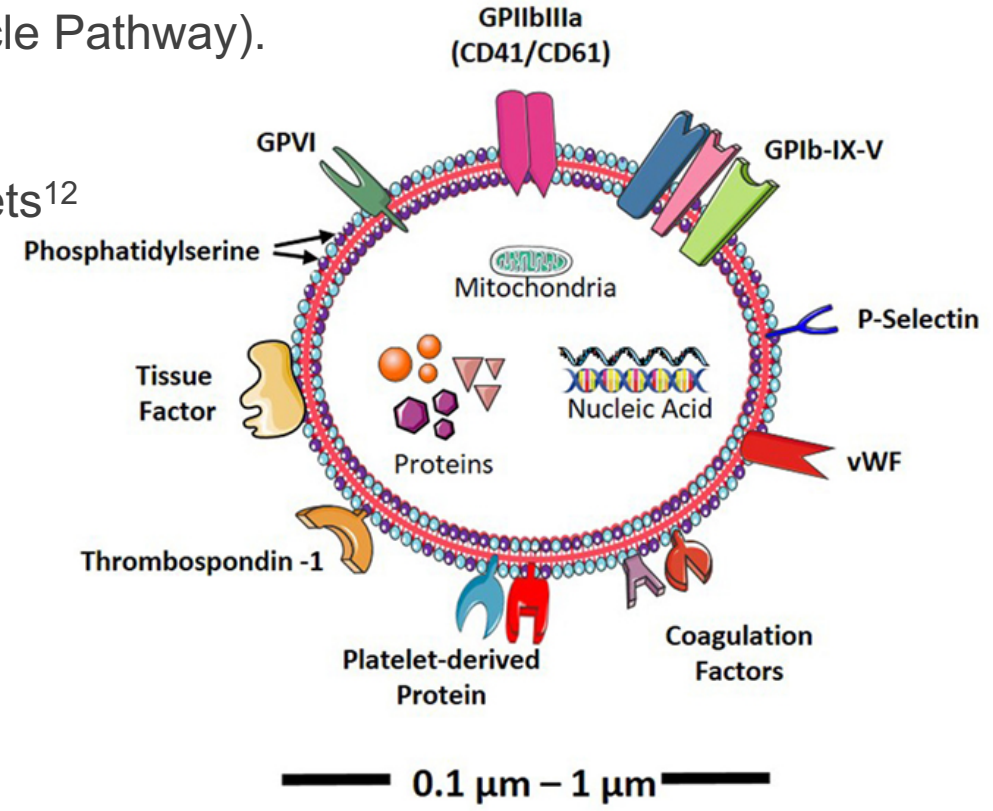


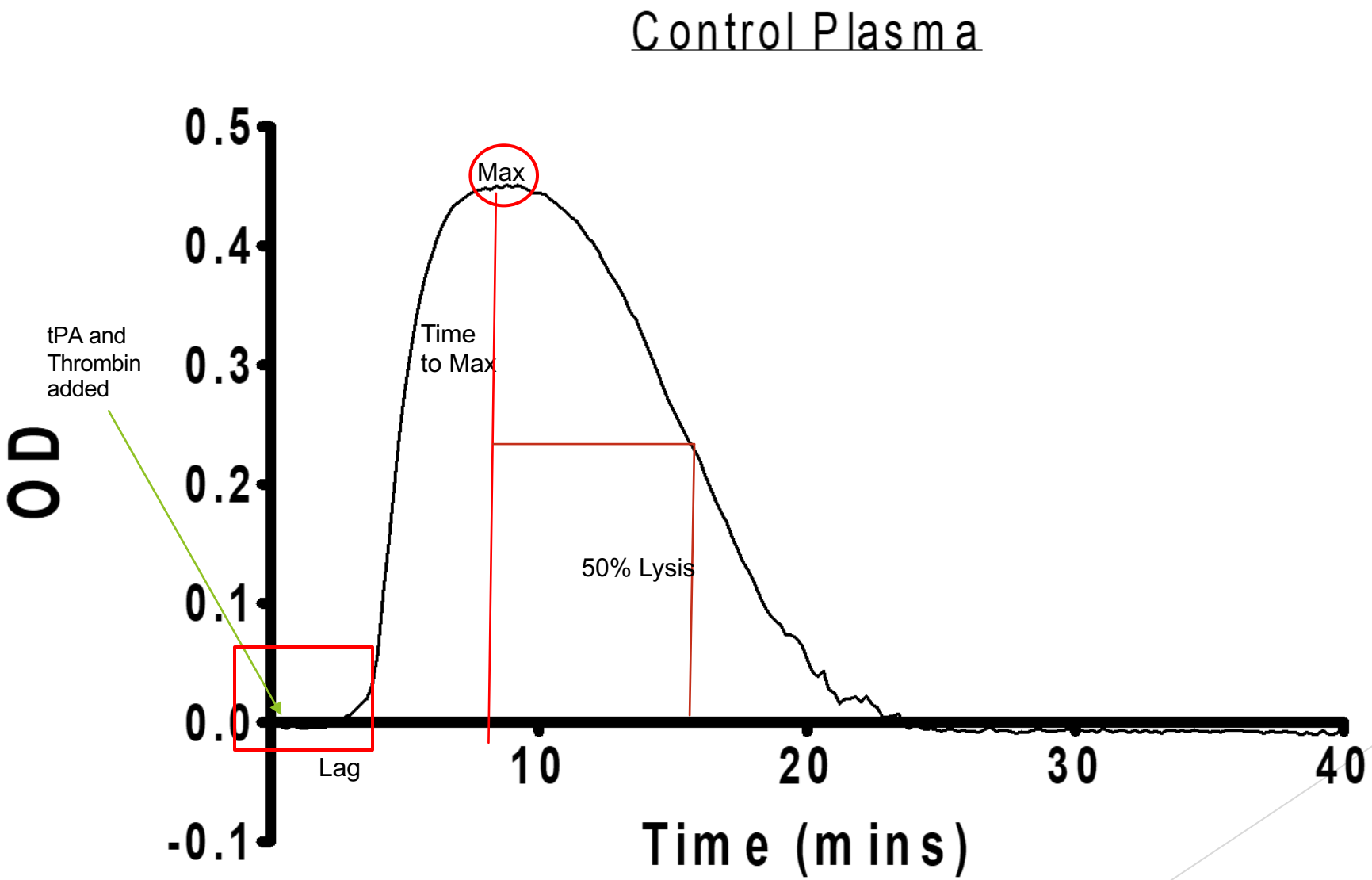
Image from - Zaldivia MTK, McFadyen JD, Lim B, Wang X, Peter K. Platelet-Derived Microvesicles in Cardiovascular Diseases. Front Cardiovasc Med. 2017;4:74.

EVs and Storage

- Increase During Storage[6,13]
- Shown to contain respiratory competent mitochondria[14-16]
- Can lead to inflammation by means of damage-associated molecular pattern (DAMPs)
  - Adverse reactions higher in those units with mitochondrial positive EVs[17].

- Study to investigate the pro-coagulant capabilities of EVs over standard storage.
  - Using Control Pooled Plasma
  - Fixed Number of EVs ($1x10^{10}$)

Turbidity and Lysis.



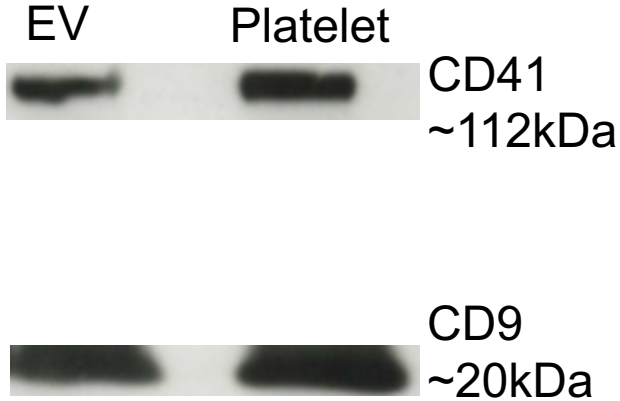
EVs, Clot formation and Lysis

Preliminary Data

Sample	Lag (s)	OD Max	Time to OD Max (mins)	50% Lysis (mins)
Control Plasma	249.00	0.457	10.25	8.40
Day 2	203.00**	0.401	7.12***	12.60**
Day 4	208.33**	0.388	7.20***	13.07***
Day 6	212.33**	0.406	7.27***	12.40**
Day 8	213.67*	0.407	7.16***	11.67**
Day 10	197.67***	0.430	6.83***	11.93**

** = P<0.05, compared to Control*
*** = P<0.01, compared to Control*
**** = P<0.001, compared to Control*
N=3

Western Blot confirming EVs are of Platelet origin



Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

Summary

PSL causes a decrease in component quality over storage

EV testing in platelet storage is a relatively new aspect for concentrate quality

PEVs are significantly pro-coagulant, strengthening the fibrin clot

Future research to investigate the effects of different storage conditions (Temperature, Oxygen) on the PSL.

Thanks For Listening

- Research is funded by KESS2 –
  - *Knowledge Economy Skills Scholarships (KESS 2) is a pan-Wales higher level skills initiative led by Bangor University on behalf of the HE sector in Wales. It is part funded by the Welsh Government's European Social Fund (ESF) convergence programme for West Wales and the Valleys.*

Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

kess2

Ysgoloriaethau Sgiliau Economi Gwybodaeth
Knowledge Economy Skills Scholarships

UNDEB EWROPEAIDD
EUROPEAN UNION

Llywodraeth Cymru
Welsh Government

Cronfa Gymdeithasol Ewrop
European Social Fund

British Blood Transfusion Society | #BBTS2019

Welsh Blood Service

References

1. Devine DV, Serrano K. The platelet storage lesion. Clin Lab Med. 2010;30(2):475-87.

2. Perrotta P, Snyder E. Platelet Storage and Transfusion. Platelets. 2007;2:1265-95

3. Böck M, Rahrig S, Kunz D, Lutze G, Heim MU. Platelet concentrates derived from buffy coat and apheresis: biochemical and functional differences. Transfus Med. 2002;12(5):317-24.

4. Dekkers DW, De Cuyper IM, van der Meer PF, Verhoeven AJ, de Korte D. Influence of pH on stored human platelets. Transfusion. 2007;47(10):1889-95.

5. Dijkstra-Tiekstra MJ, Pietersz RN, Huijgens PC. Correlation between the extent of platelet activation in platelet concentrates and in vitro and in vivo parameters. Vox Sang. 2004;87(4):257-63.

6. Sandgren P, Saeed K. Storage of buffy-coat-derived platelets in additive solution: in vitro effects on platelets of the air bubbles and foam included in the final unit. Blood Transfus. 2011;9(2):182-8.

7. Black A, Pienimaeki-Roemer A, Kenyon O, Orsó E, Schmitz G. Platelet-derived extracellular vesicles in plateletpheresis concentrates as a quality control approach. Transfusion. 2015;55(9):2184-96.

8. Morelli AE, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood. 2004;104(10):3257-66.

9. Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. Exp Cell Res. 2009;315(9):1584-92.

10. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol. 2013;113(1):1-11.

11. Fox JE, Austin CD, Boyles JK, Steffen PK. Role of the membrane skeleton in preventing the shedding of procoagulant-rich microvesicles from the platelet plasma membrane. J Cell Biol. 1990;111(2):483-93.

12. He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8(1):237-55.

13. Flaumenhaft R. Formation and fate of platelet microparticles. Blood Cells Mol Dis. 2006;36(2):182-7.

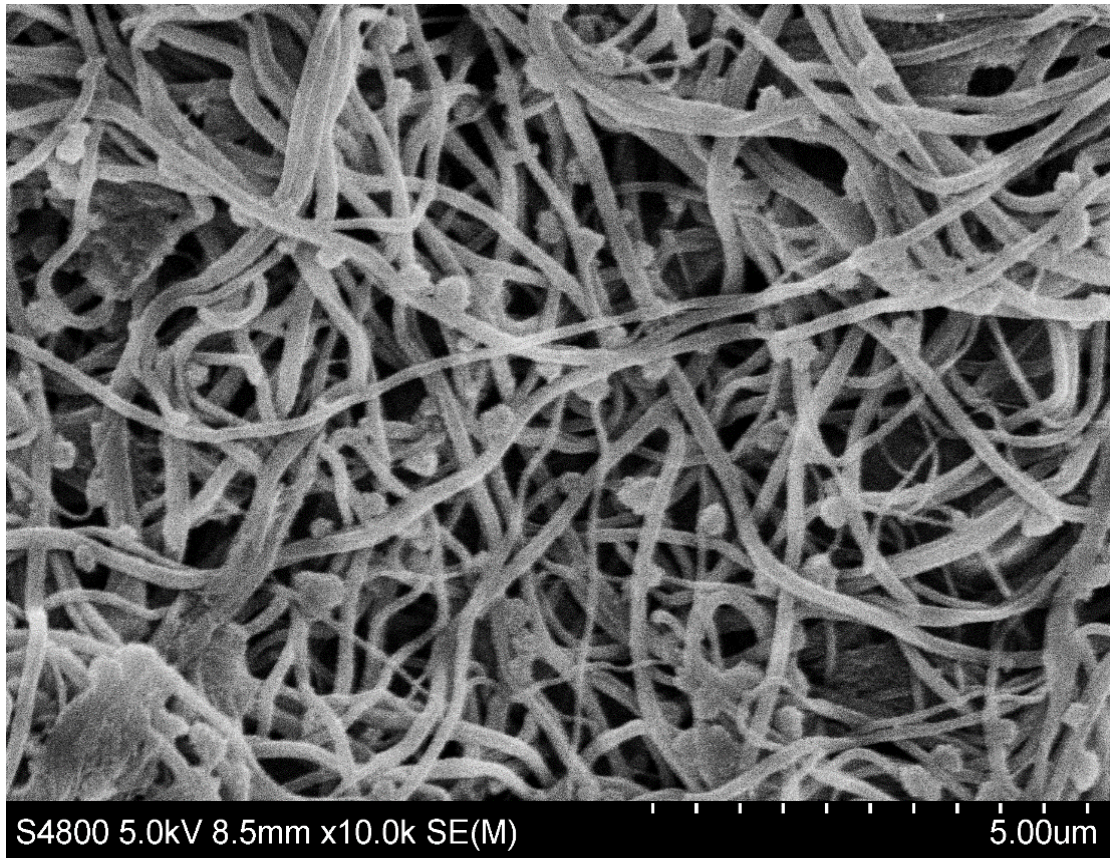
14. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. Blood. 2014;124(14):2173-83.

15. Marcoux G, Duchez AC, Rousseau M, Lévesque T, Boudreau LH, Thibault L, et al. Microparticle and mitochondrial release during extended storage of different types of platelet concentrates. Platelets. 2017;28(3):272-80.

16. Chen Z, Schubert P, Bakkour S, Culibrk B, Busch MP, Devine DV. p38 mitogen-activated protein kinase regulates mitochondrial function and microvesicle release in riboflavin- and ultraviolet light-treated apheresis platelet concentrates. Transfusion. 2017;57(5):1199-207.

17. Marcoux G, Magron A, Sut C, Laroche A, Laradi S, Hamzeh-Cognasse H, et al. Platelet-derived extracellular vesicles convey mitochondrial DAMPs in platelet concentrates and their levels are associated with adverse reactions. Transfusion. 2019;59(7):2403-14.

Supplementary Material



EM image of a fibrin clot structure after the addition of EVs