Frysta trombocyter: från traditionella frysmedier till framtidens metoder

Per Sandgren

Conflict of Interest Statement

The author declares that there are no conflicts of interest related to this presentation.

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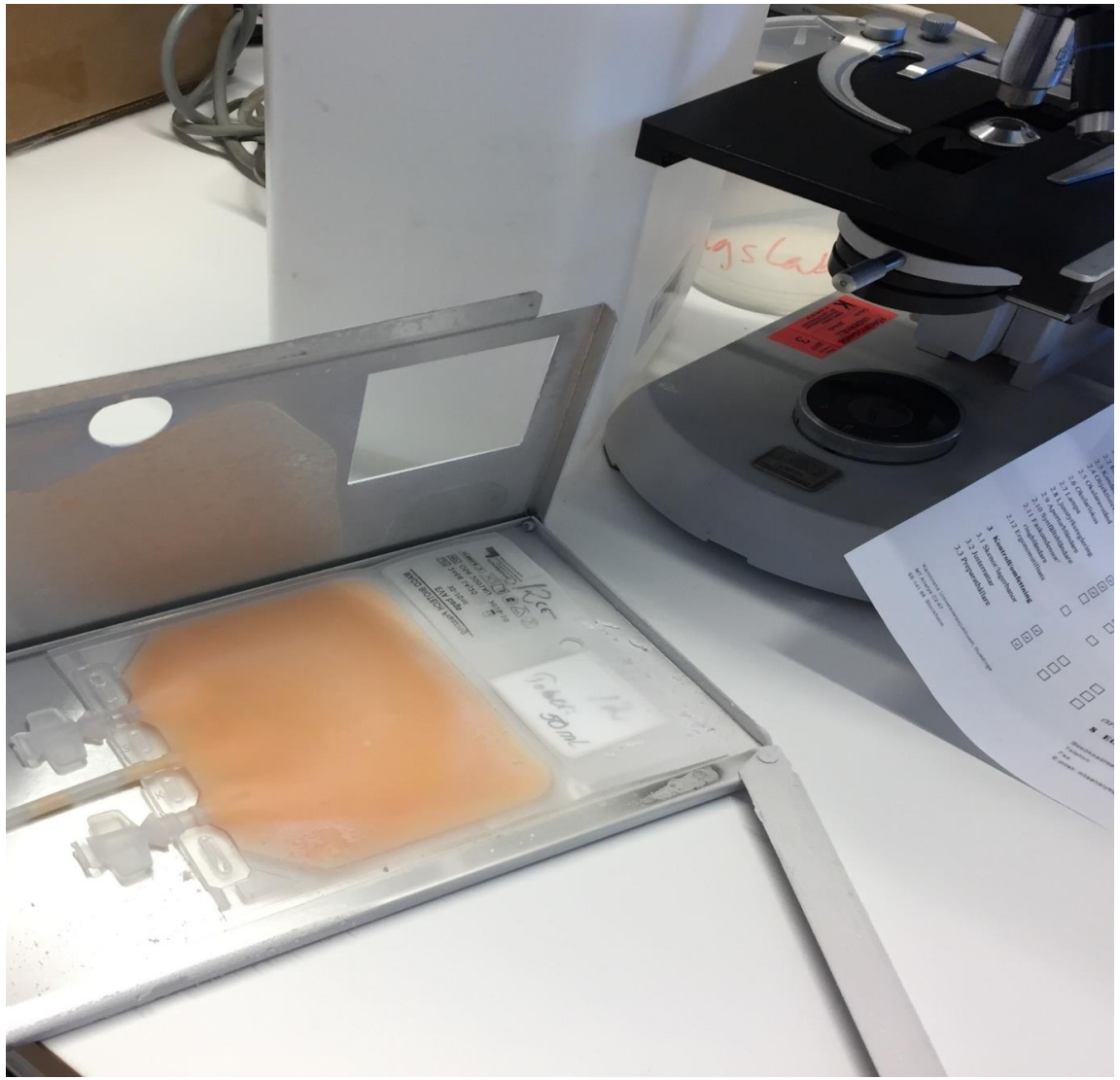
FRYSTA TROMBOCYTER, bakgrund

- Bidrar till att täcka bristsituationer av konventionellt lagrade trombocyter
- Kan fungera som reservlager i krissituationer
- Tillgängliggör trombocyter på avlägsna platser
- Möjliggör infrysning av sällsynta fenotyper
- Frysta trombocyter lagras i en liten volym, 5% DMSO (frysmedium)
- Kan förvaras upp till 4 år
- Tinas och rekonstrueras i färsk kompatibel plasma
- Transfunderas inom 5 timmar vid aktiv blödning



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Dimethyl sulfoxide (Me2SO; commonly referred to as DMSO)

- DMSO has been essential in preserving cells for over 50 years.
- ***It has helped enable treatments for thousands of patients worldwide***.
- First shown to capable of mitigating freezing-related cell injuries (Nature 1959).

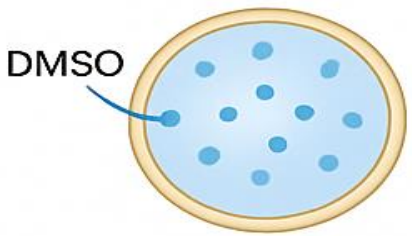
- Today, research focuses on reducing side effects and finding safer options.
- These approaches include:
  - *Adding other cryoprotectants (CPA)* e.g; sugars, polymers, etylenglykol, propylenglykol, polyvinylpyrrolidon (PVP), hydroxyetylstärkelse (HES)
  - *Using alternatives like*; Glycerol, Trehalose, Carboxylated Poly-L-Lysine, Commercial DMSO-Free Media (e.g., Bambanker DMSO-Free)
  - *Developing novel DMSO/CPA-free methods*

Hypothesis of Platelet Cryoprotection With DMSO

How DMSO Protects Platelets During Cryopreservation

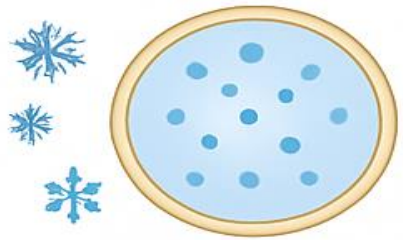
Panel 1: DMSO Permeation

DMSO rapidly enters platelets, distributing between intra- and extracellular spaces



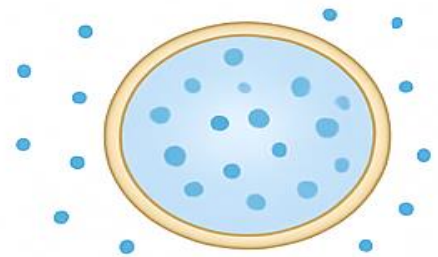
Panel 2: Ice Growth Inhibition

DMSO interferes with water molecule orgamization, reducing extracellular and intracellular ice crystal formiton



Panel 3: Osmotic Bürriering

DMSO presence moderates osmotic stress during cooling and thawing, preventing

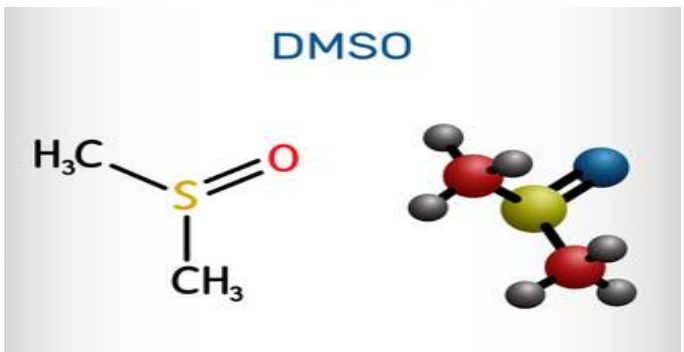


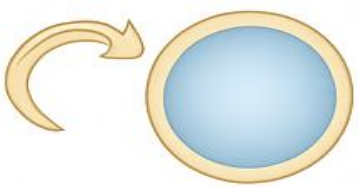
Panel 4: Membrane Protection

DMSO stabilizes lipid bilayers by intercalating into inembranes, maintaining fluidity and preventing rupture

Panel 5: Protein Stabilization

DMSO reduces denaturation of cytoskeletal and membranes, maintaining platelet integrity





DMSO permeates cells ⟶ Balances iintra/extracelluiar environment

Inhibits ice nucleation and growth ⟶ Prevents intracellular damage

Buffers osmotic stress ⟶ Reduces volume shock

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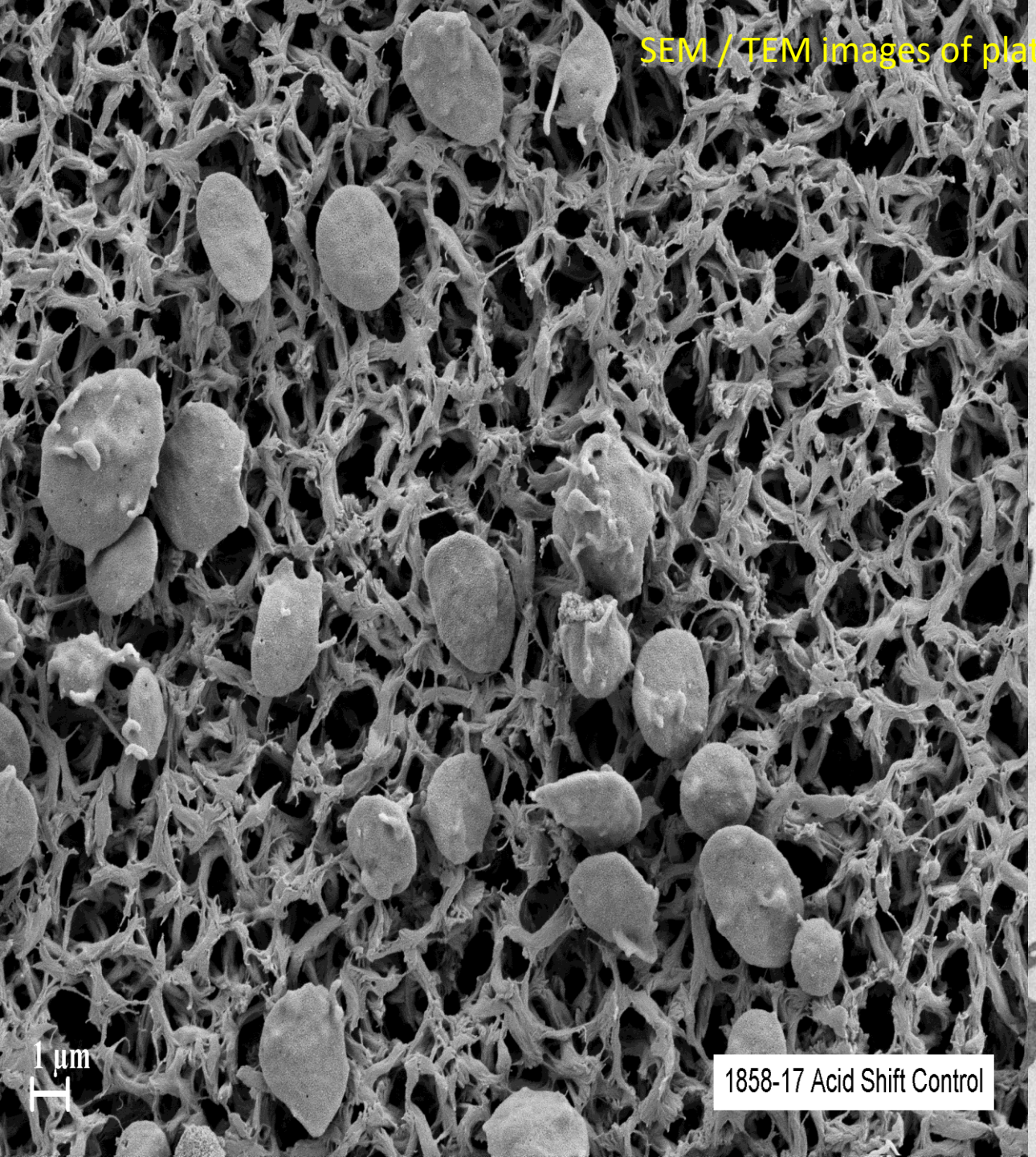
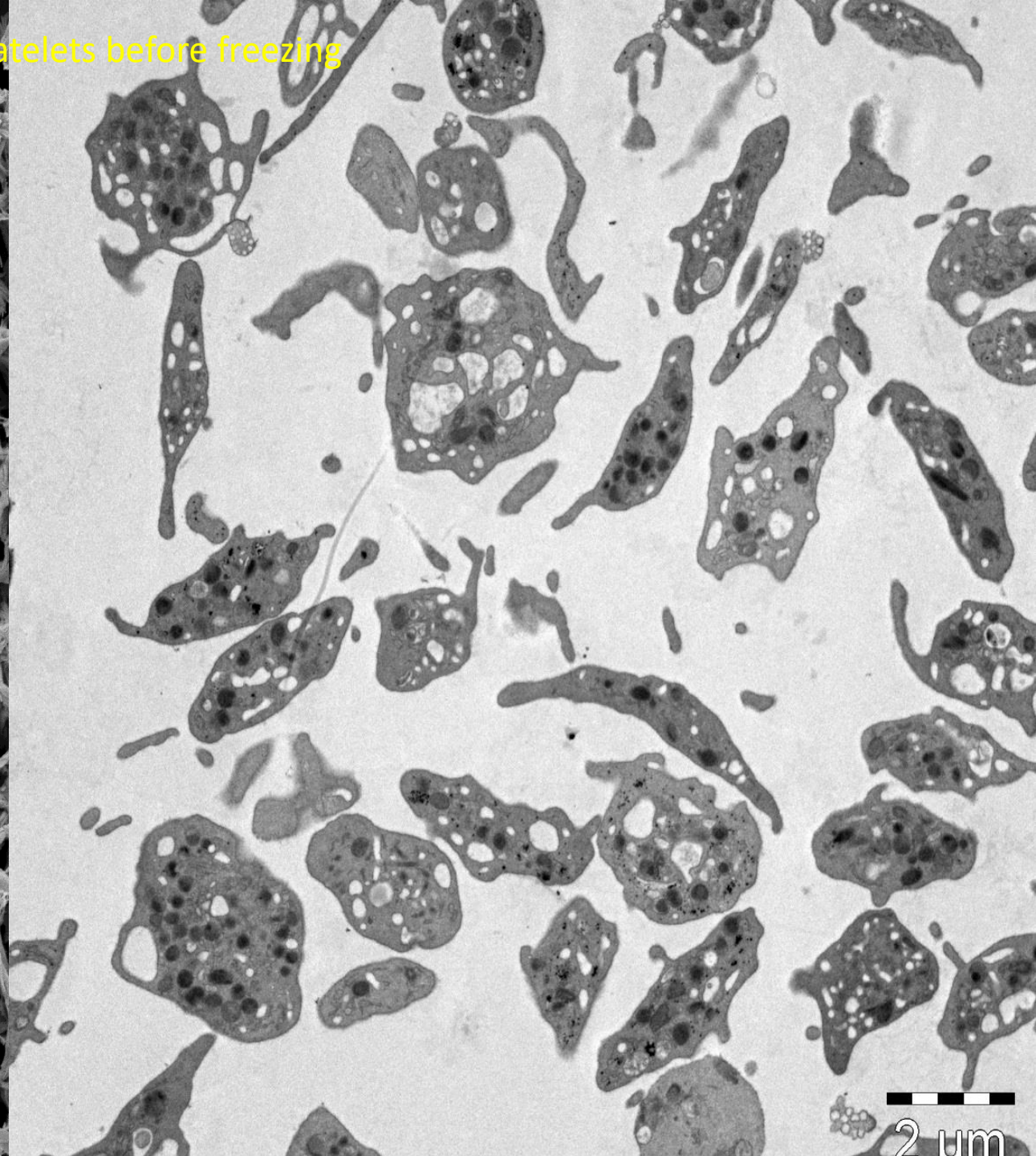
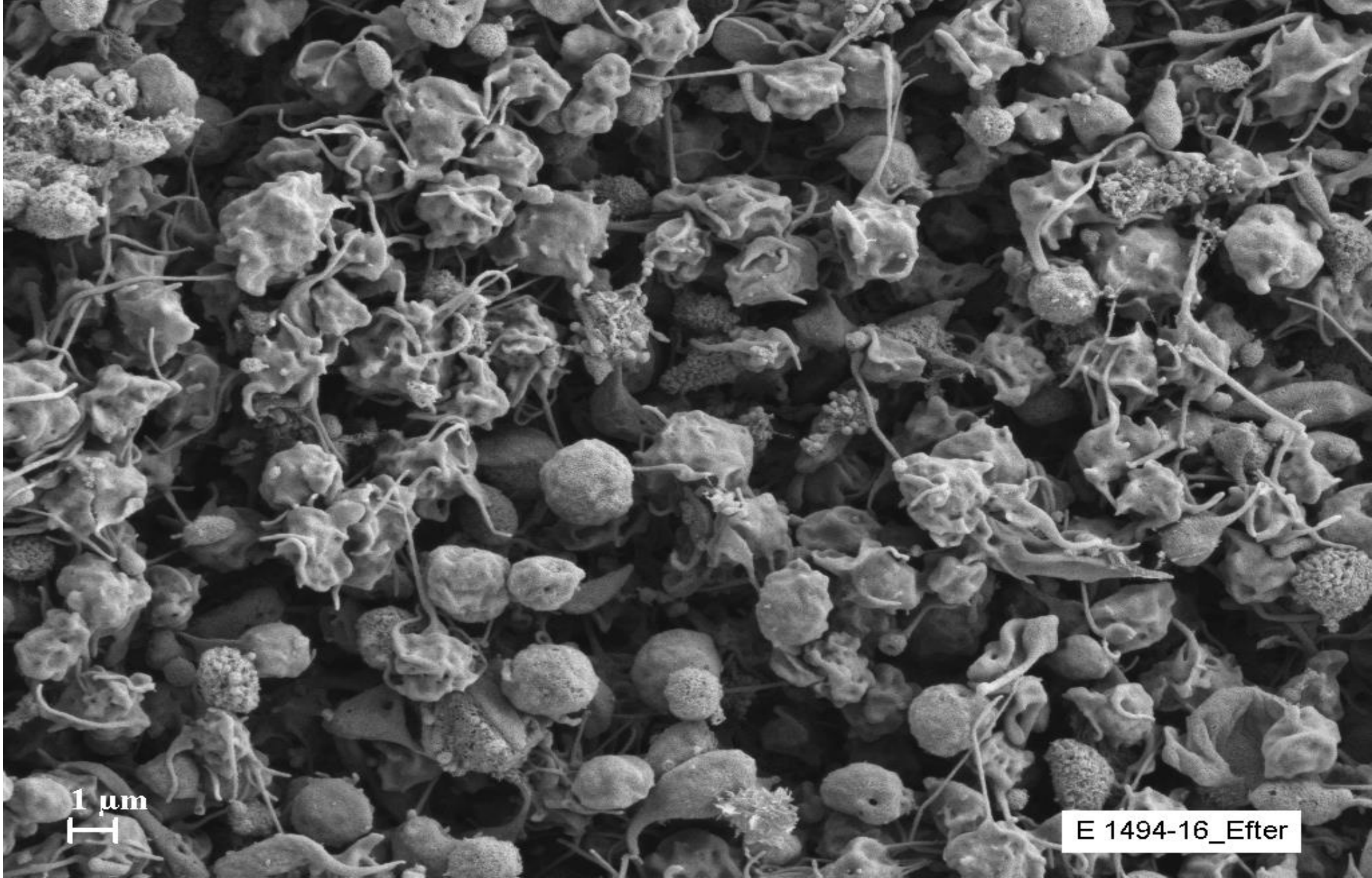
SEM / TEM images of platelets before freezing

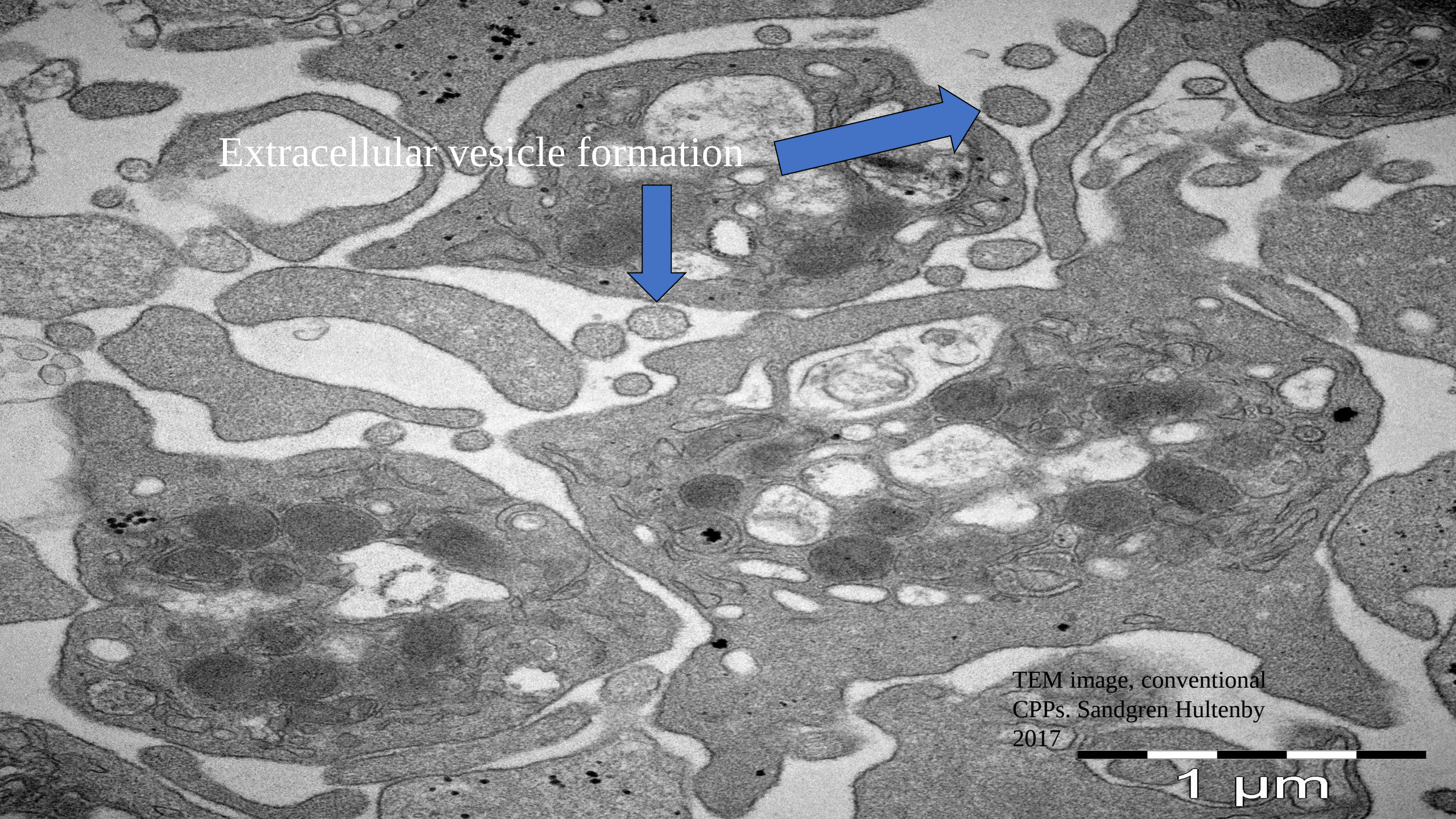




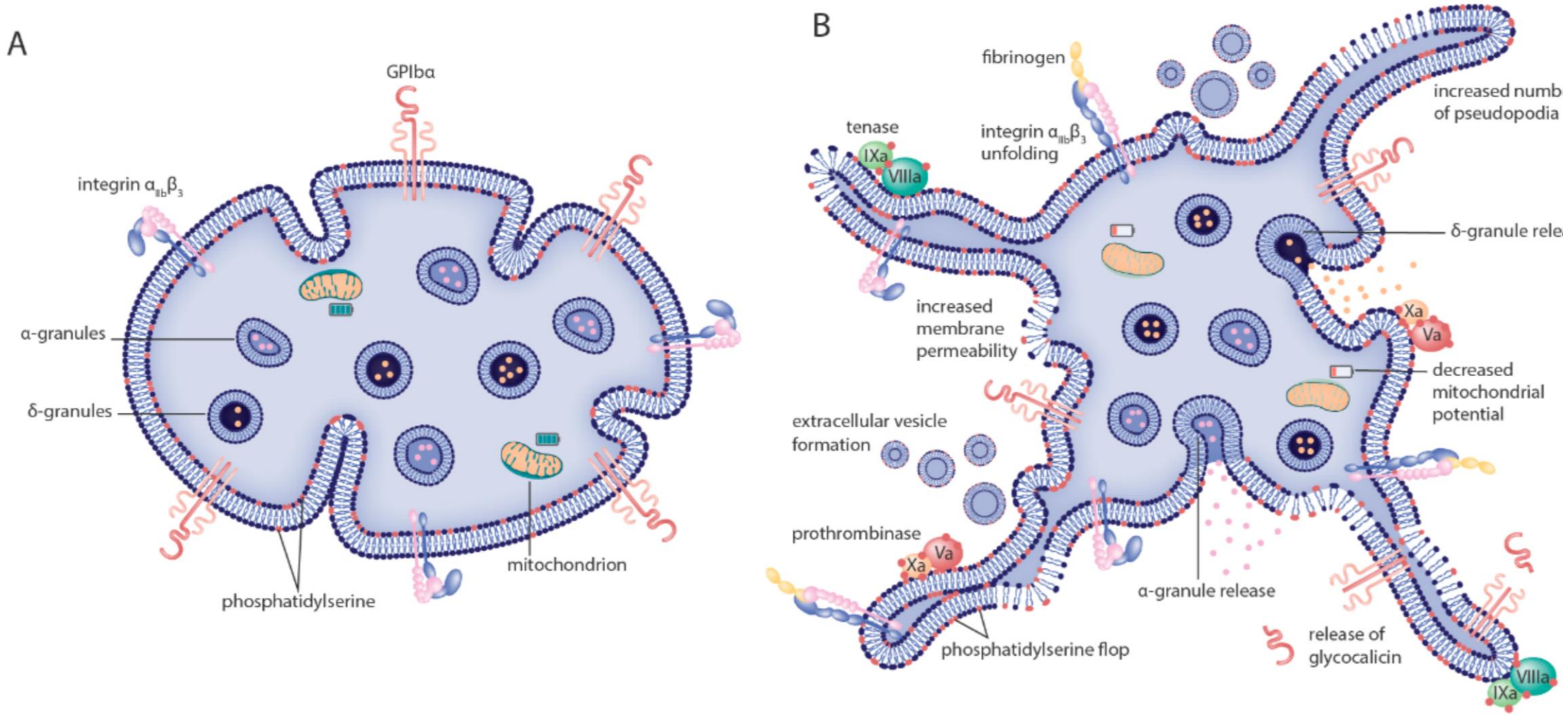
SEM image of platelets in DMSO after freezing and thawing



P Sandgren and K Hultenby


Extracellular vesicle formation
TEM image, conventional CPPs. Sandgren Hultenby 2017
1 µm

Platelet Biochemistry and Morphology after Cryopreservation



Six et.al. Int. J. Mol. Sci. 2020, 21(3), 935;

Project Aims

investigate and compare the cellular effects such as recovery, metabolic and apoptotic activity, phenotype expression and in vitro functionality on platelets after cryopreservation **comparing a novel freezing approach with conventional freezing of platelets by using DMSO as cryoprotectant.**

develop and finalize a clinical protocol generating a completely non-toxic frozen platelet product aimed for transfusion

Key findings and methods from this study

Freezing protocol:

- Platelets were frozen in a high volume of NaCl solution without traditional cryoprotectants like DMSO.
- In addition, we also investigated a specific freezing protocol involving gradual cooling and controlled freezing (CRF).

Results and observations:

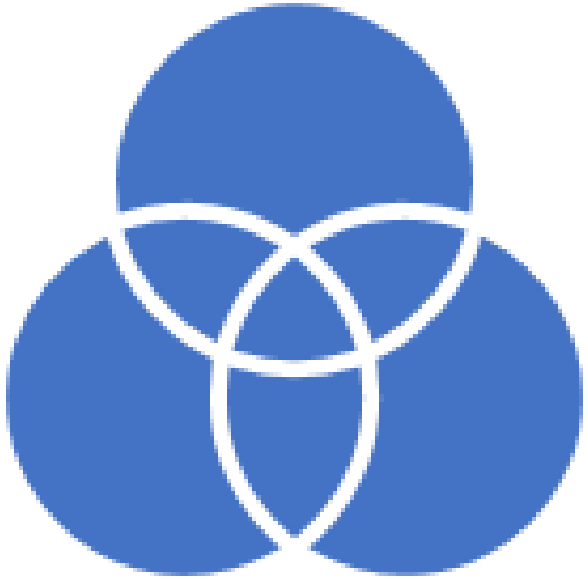
- NaCl Improved recovery of cells (90%) as compared to DMSO frozen platelets (60%)
- NaCl-platelets show hemostatic function in vitro.
- NaCl platelets retained important phenotypic structures, crucial for their role in the coagulation process.
- All platelets release a significantly increased amount of platelet-specific microparticles.
- CRF improve in vitro data

Potential for clinical use:

- The study indicates that freezing with only NaCl solution may be a feasible alternative for cryopreservation of platelets, potentially simplifying and reducing costs of the process.
- This could lead to less toxic methods of cryopreservation and possibly better compatibility for transfusions.

Ehn, K.; Wikman, A.; Uhlin, M.; Sandgren, P. Cryopreserved Platelets in a Non-Toxic DMSO-Free Solution Maintain Hemostatic Function In Vitro. Int. J. Mol. Sci. 2023, 24, 13097. https://doi.org/ 10.3390/ijms241713097

Remaining key questions

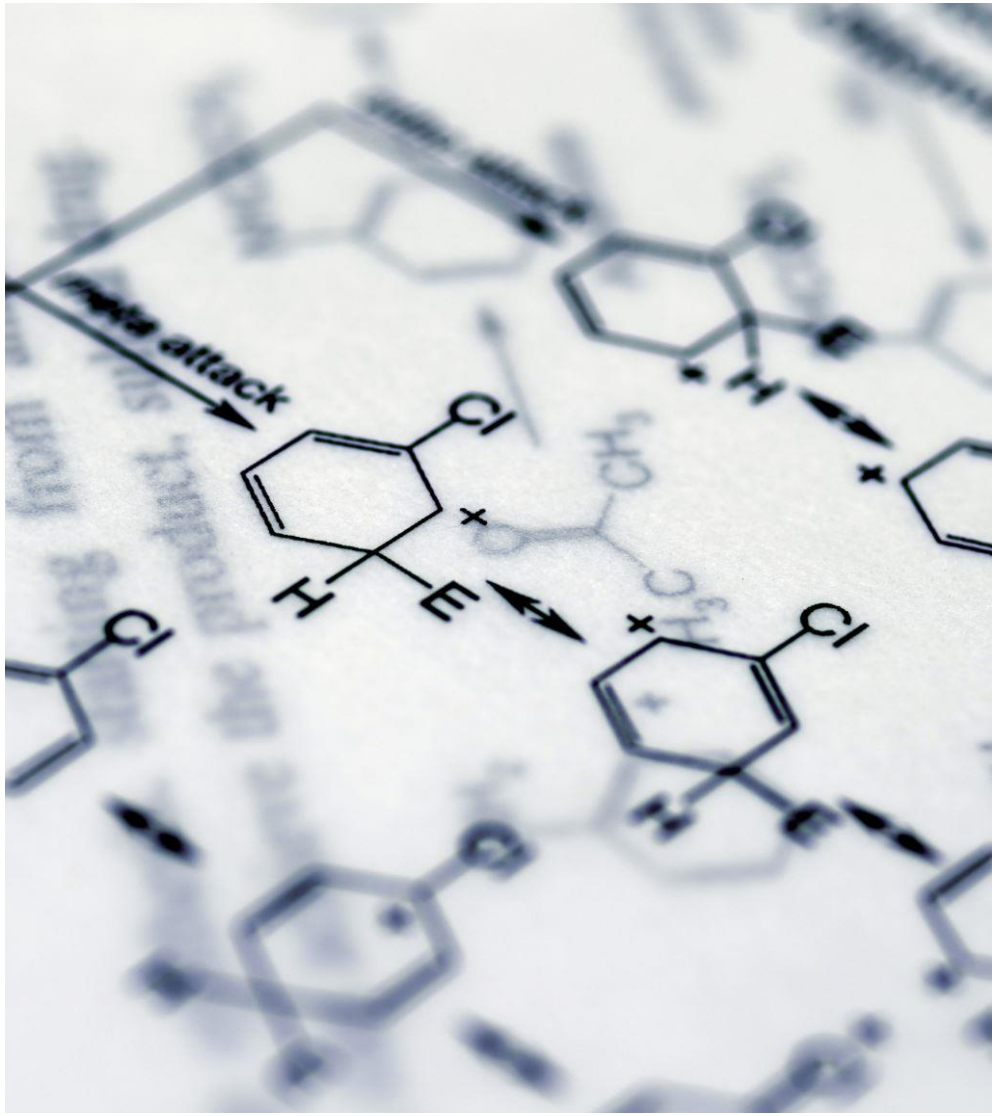


- The explanatory model is hypothetical and not supported by the presented data, further studies are desirable;
- Potential advantages/disadvantages of using hypertonic NaCl solution for freezing platelets?
- find out the biological significance of the different EVs/microparticles that are released after freezing and thawing
- investigate the possibility of including additives compatible with NaCl freezing of platelets
- *All in attempt to optimize a future protocol for DMSO-free platelets*

Deep Eutectic Solvents (DES) vid frysning av celler
Vad är DES?

DES bildas av två eller flera komponenter, t.ex. prolin och glycerol, vilket ger en lösning med låg smältpunkt och god biokompatibilitet.

- *Funktion som kryoprotektant*
  - *DES skyddar celler vid frysning genom att binda vattenmolekyler, minska isbildning och därmed reducera mekaniska skador på cellmembran.*
  - *De har lägre toxicitet jämfört med traditionella ämnen som DMSO och glycerol, vilket gör dem attraktivare för kliniska applikationer.*
  - *Effekt och fördelar*
  - *DES möjliggör hög cellviabilitet efter upptining (t.ex. över 94% för stamceller).*
  - *Kan användas direkt utan behov av att avlägsna dem efter upptining, vilket förenklar processen.*



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Highlighted DES: Choline chloride (10%) : Glycerol (Glyceline, 1:3)

• Low toxicity • Strong H-bonding • Ice growth suppression

Protein/Receptor Stabilization
- Preserves GPIb, GPIIb/IIIa
- Reduces receptor loss
- Maintains signaling proteins

Membrane & Cytoskeleton
- Lipid bilayer stabilization
- Cytoskeletal protection
- Reduced membrane rupture

Functional Preservation
- Better post-thaw aggregation
- Improved clot retraction
- Maintains hemostatic function

Osmotic Balance
- Controlled dehydration
- Reduced osmotic stress
- Slows water flux

Water/Ice Control
- Freezing point depression
- Ice recrystallization suppression

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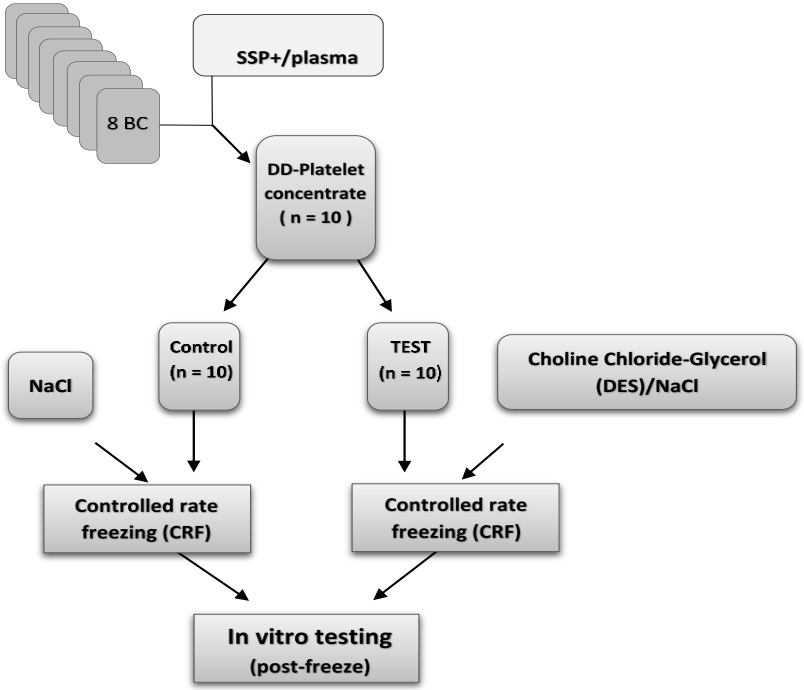
Innovations in Platelet Cryopreservation: Evaluation of DMSO-Free Controlled Rate Freezing and the Role of a Deep Eutectic Solvent as Additional Cryoprotective Agent

R Befekadu[2], N Bosnjak[2] M Uhlin[1], A Wikman[1, 2] and P Sandgren [1, 2]
[1]Hematology and regenerative medicine (HERM), Karolinska Institutet, Stockholm, [2]Clinical immunology and transfusion medicine (KITM), Karolinska University Hospital, Huddinge, Sweden

- **Material and Methods**
- Analysis of cell count, recovery, disintegration (LDH % of total) and blood gas
- **Flow Cytometry Analysis**: P-selectin (CD62P) (CD63**)**: (CD42b**)** (CD61)(CD41a**)** GPVI: PECAM-1 (CD31):TLR2 and TLR4 (Toll-like receptor 2 and 4): lysosomal-associated membrane protein (LAMP-1) :Mitochondrial membrane potential (Δψm) Microparticles (MPs) CD61, TLR2, TLR4, and GPVI
- **Thromboelastometry** (Rotem/TEG)

Figure 1. Schematic overview of the paired two-armed study design.



- *Appendix A.1* ***Comparative Effects of Uncontrolled Platelet Freezing in iso and hypertonic NaCl***

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Take home message!

Frysta trombocyter i DMSO räddar liv!

Dock är DMSO inte ett optimalt frysmedium

Förbättringsarbete pågår

Stort Tack till alla inblandade som engagerat sig i frysta trombocyter!

Per Sandgren