CINCINNATI RESEARCH IN TRAUMA

RBC Thromboinflammation: The Direct Red Effect (DRE)

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Disclosures

- No relevant financial disclosures
- Not a hematologist
- Don’t have a PhD
- Just a simple country trauma surgeon from the Midwest

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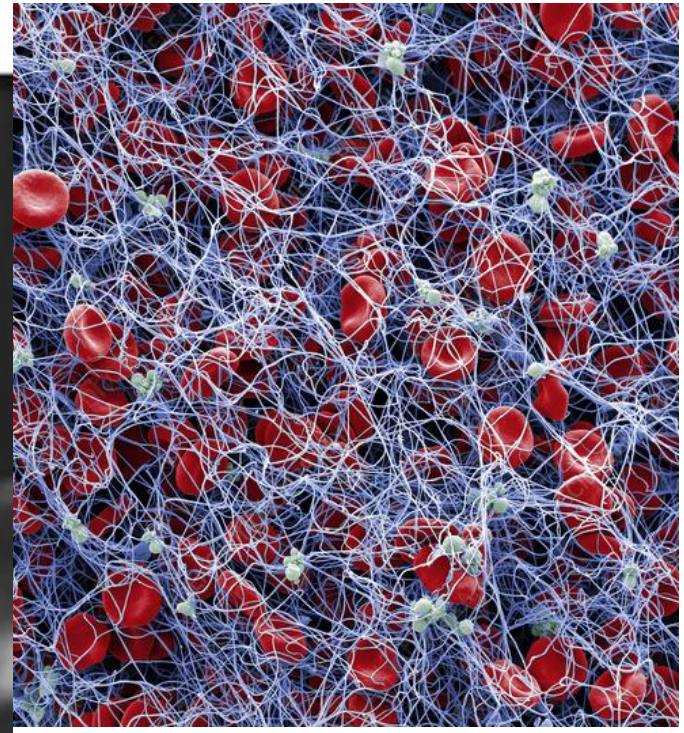
RBC Role in Thrombosis



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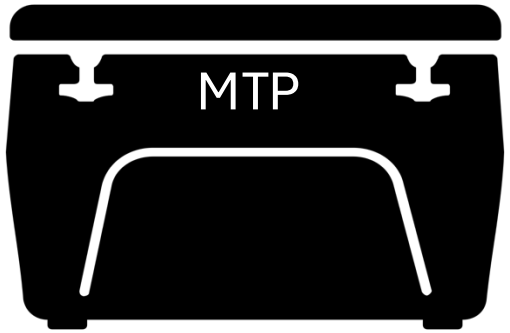
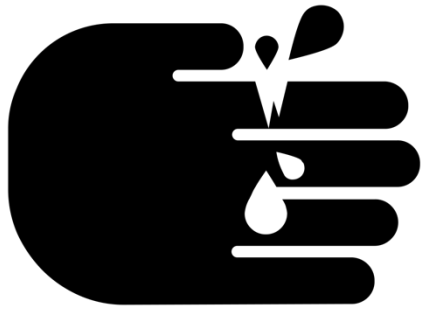
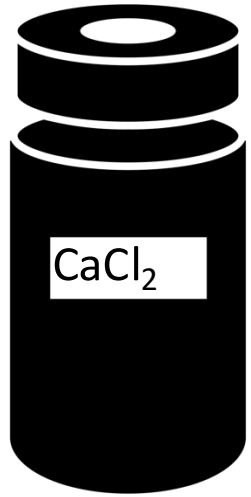
RBC Role in Thrombosis



High transfusion requirements associated with increased thromboembolic events



Microvascular injury leads to systemic rise in circulating Tissue Factor (TF)



Avoidance of hypocalcemia is a critical component of blood product-based resuscitation

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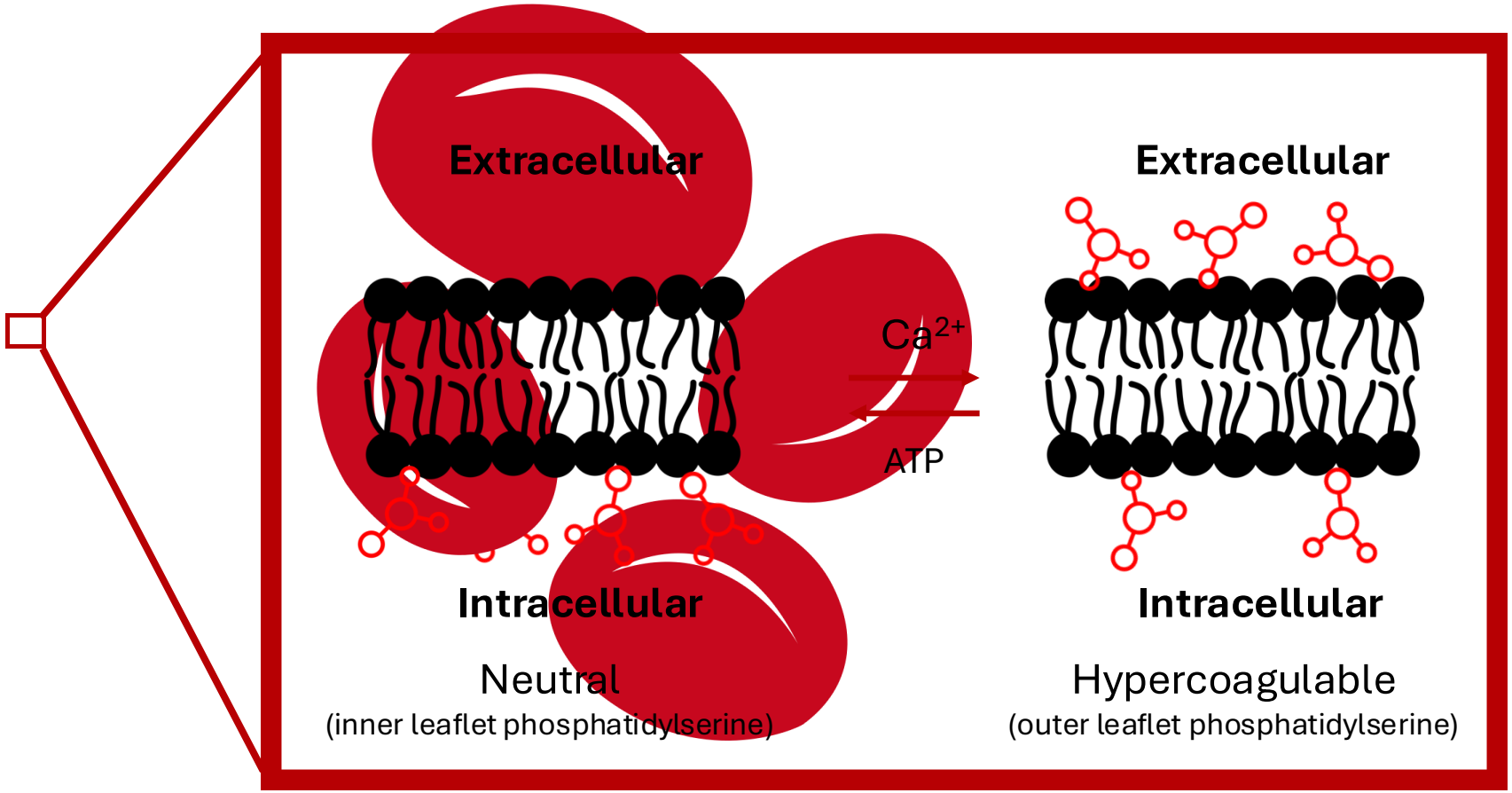
RBC Membrane Phospholipid Asymmetry



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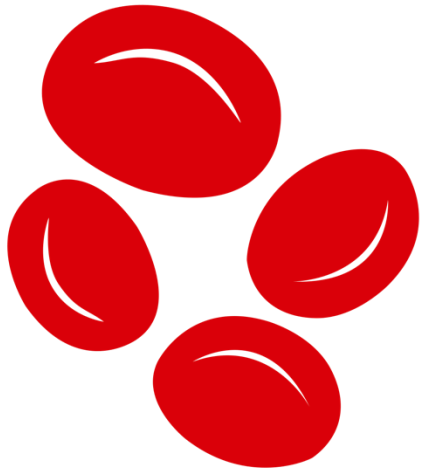
Initial Aim



Acute Trauma:
Tissue Factor
Hypercalcemia

? Pathologic Thrombosis
(Direct Red Cell Effect)

Determine the role of TF and Calcium in the Direct Red Blood Cell Effect on Thrombosis

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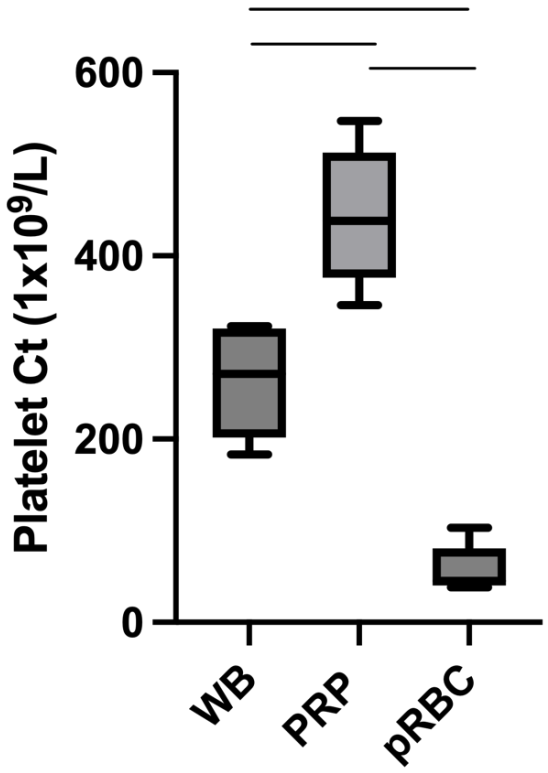
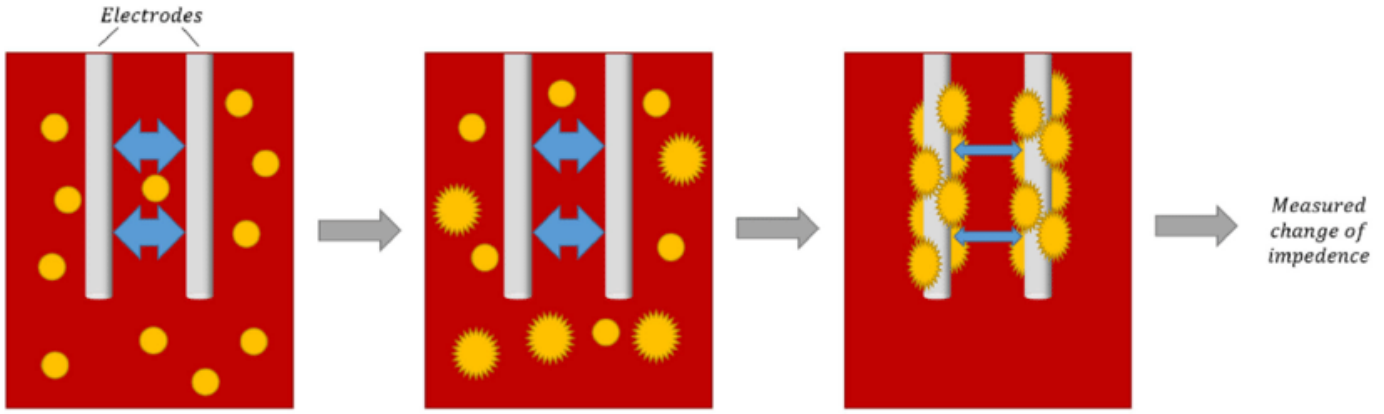
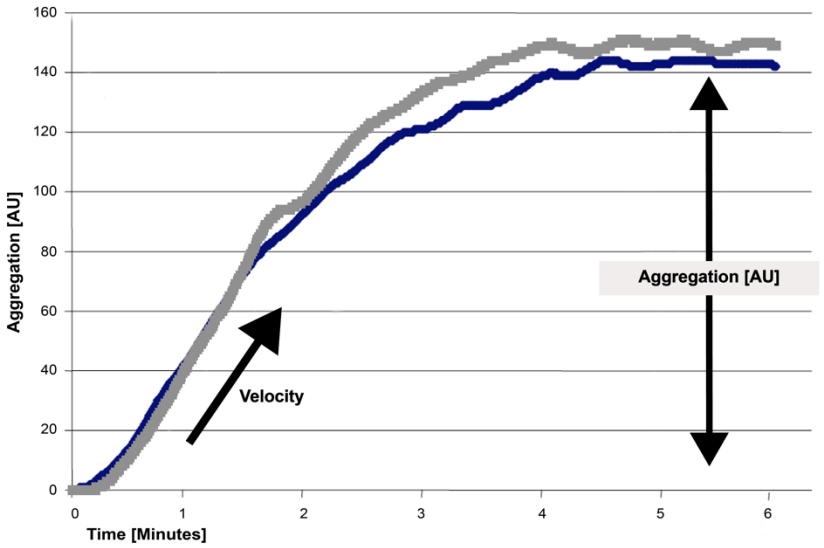
Methods - RBC Isolation and Storage

- Fresh **whole blood (WB)** obtained initially from trauma patient WB samples, subsequently from healthy volunteers (storage day 0-21)
- Centrifuged at 300xG for 6 minutes, 4°C
  - Supernatant removed → **"Platelet-Rich" Plasma (PRP)**
  - Pellet including RBC, plasma salvaged
- Pellet centrifuged 1000xG for 15 minutes, 4°C
  - Supernatant removed → "Platelet-Poor" Plasma (PPP)
- Remaining pellet → **Isolated packed Red Blood Cells (pRBC)**
  - Stored in AS-3 Solution at 2:9 ratio with initial WB volume
  - AS-3 composed of saline-based adenine/dextrose solution
- Cell count confirms intended component variation
- Replicates clinical pRBC isolation



Methods - Impedance Aggregometry





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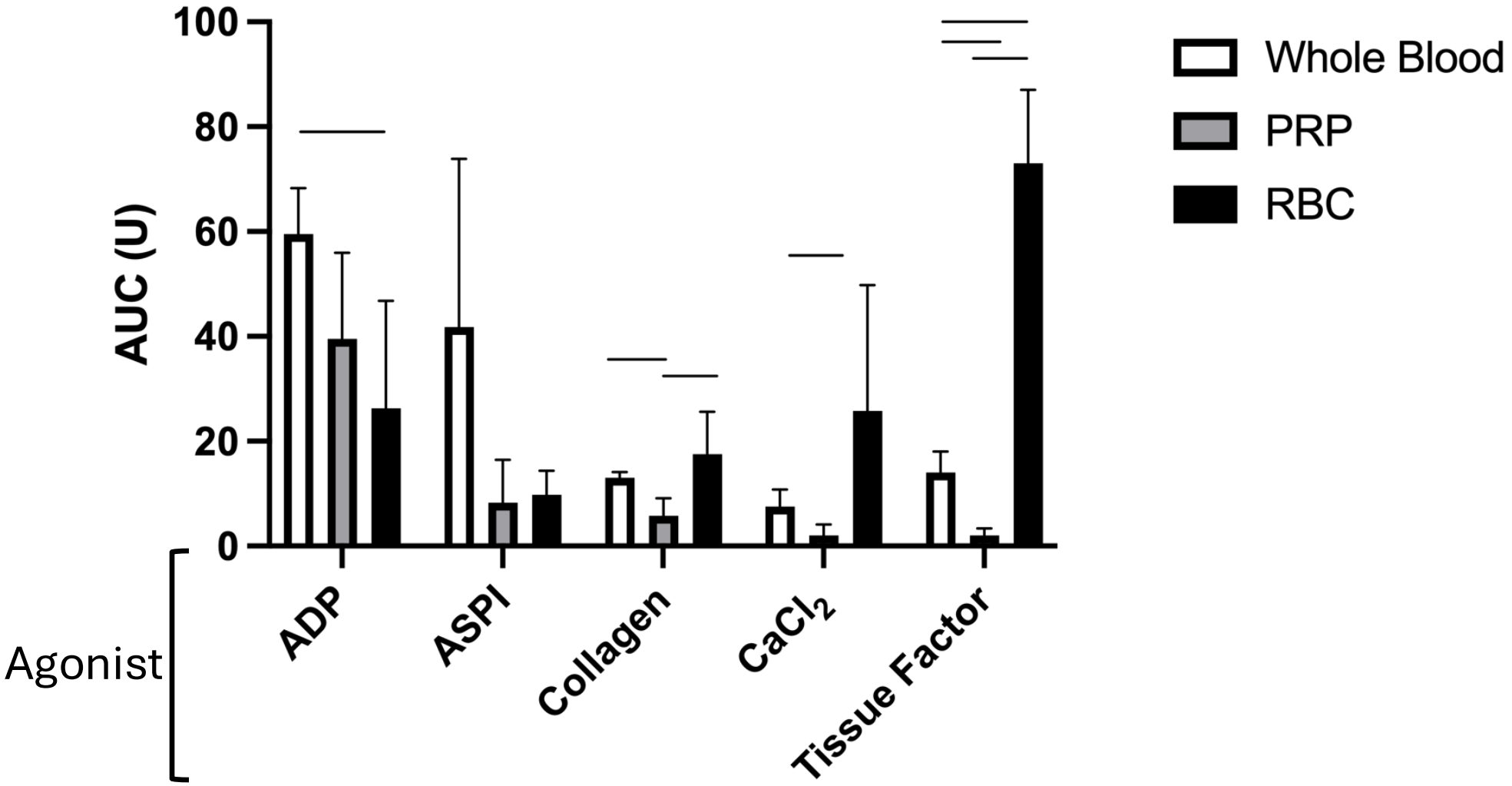
Blood Component Aggregation Analysis



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RBC Impedance Aggregometry Agonists

200mM $CaCl_2$

(in HEPES buffer (pH 7.4), 0.1% NaN_3)

Recombinant Tissue Factor

ROTEM reagent used for extrinsic activation

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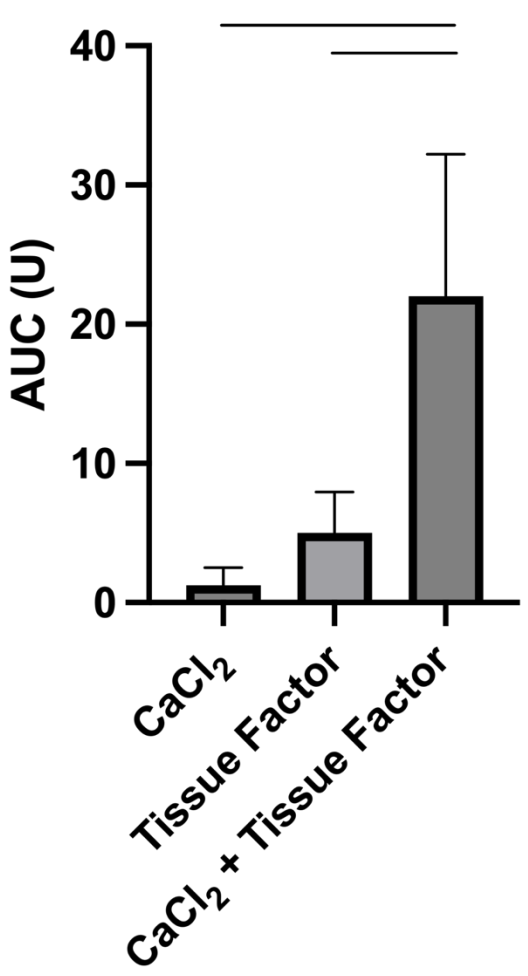
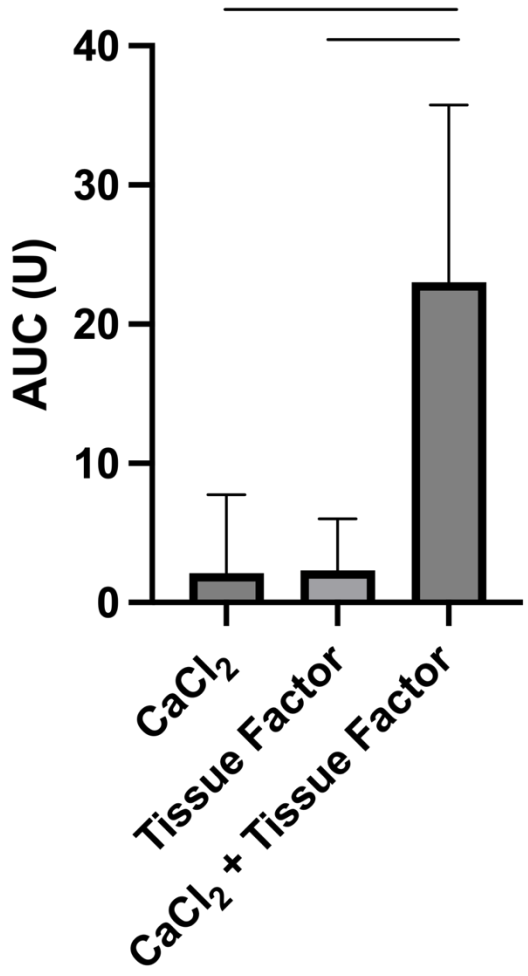
RBC Aggregation in Populations

Trauma pt
WB samples
n=9





Donated WB,
stored as pRBC
21 days
n=4

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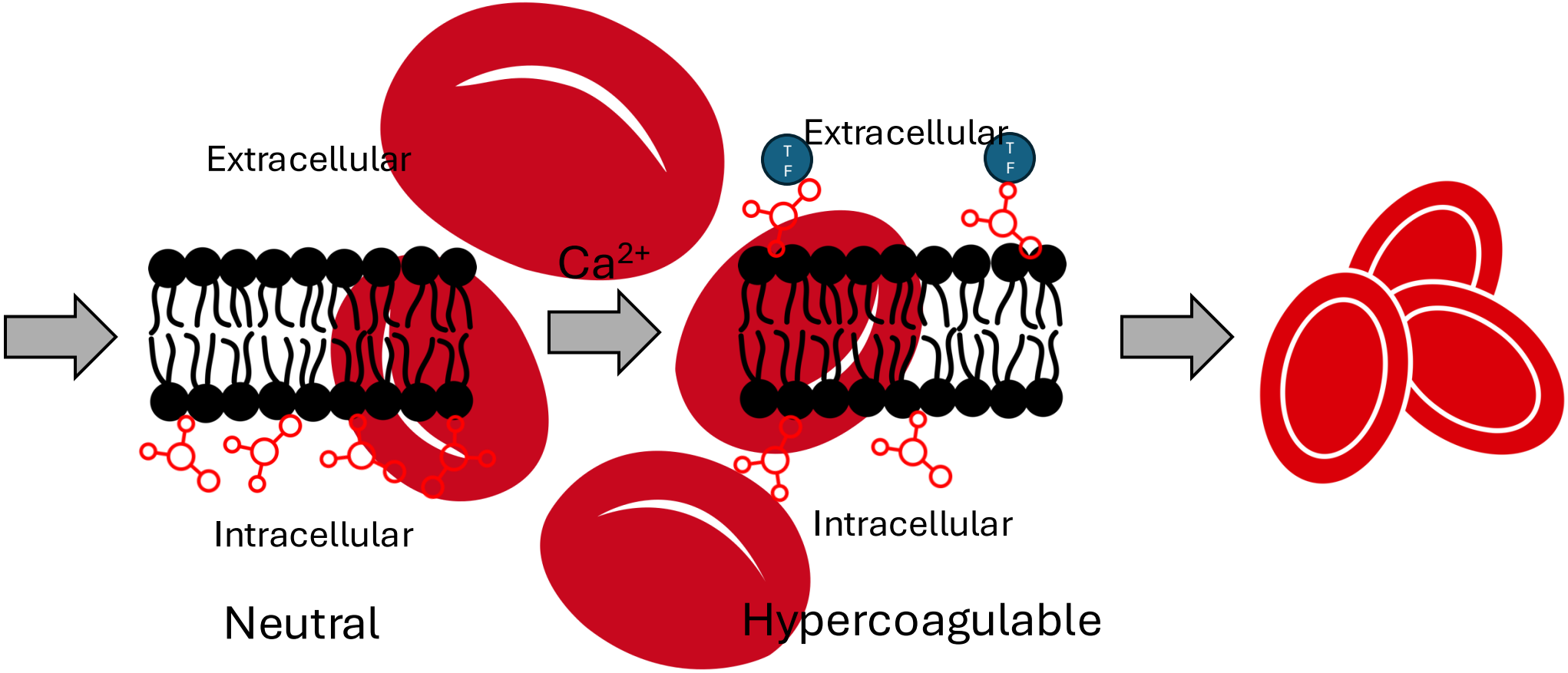
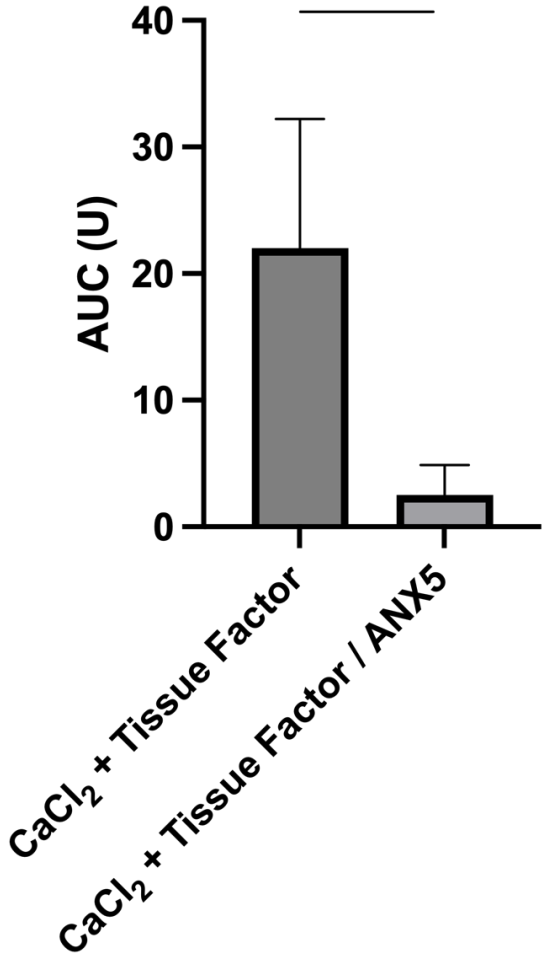
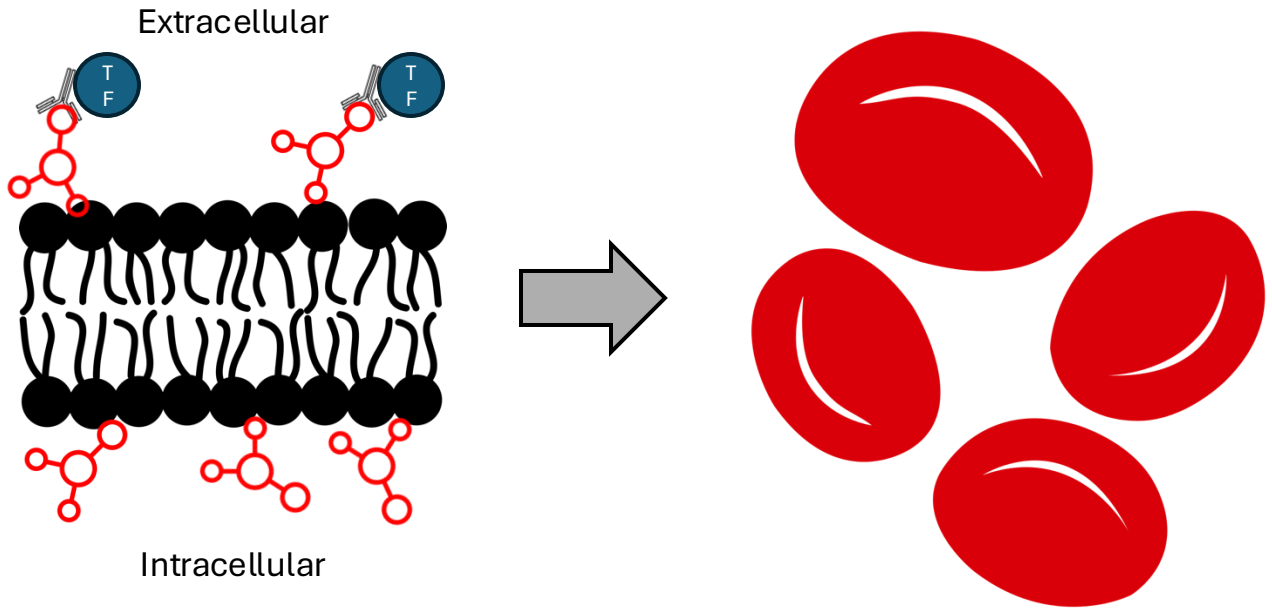
RBC Aggregation Requires Calcium and Tissue Factor



RBC Aggregation Inhibited by PS Blockade





"Hypercoagulable"
+ PS blockade

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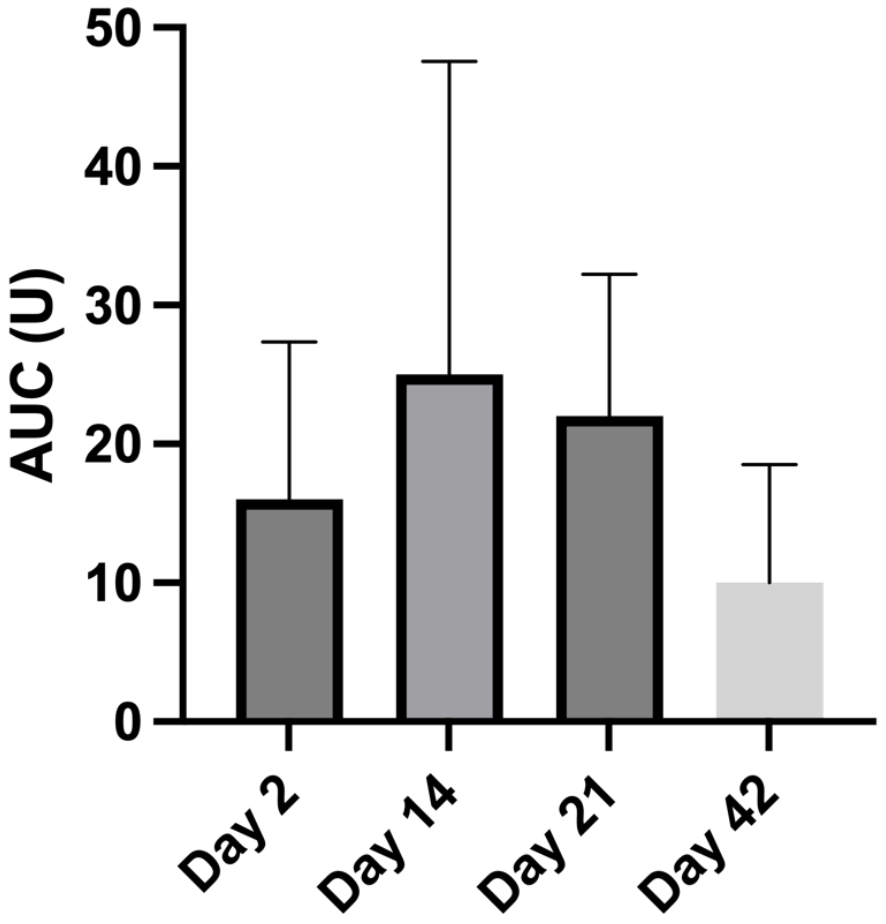
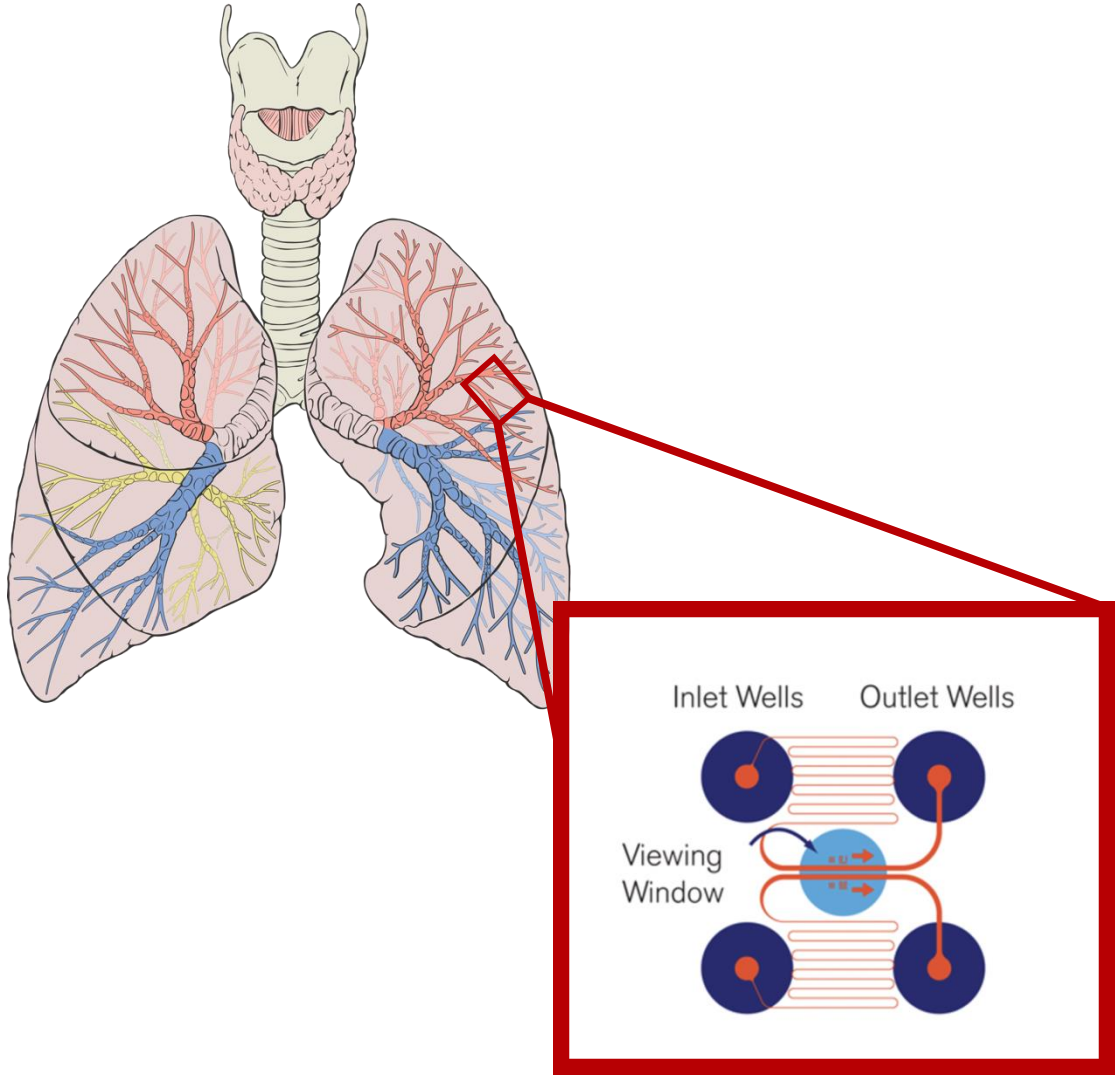
RBC Aggregation by Product Age



Pulmonary Microcirculatory Perfusion

- Microfluidic channel cultured with Pulmonary Artery Endothelial monolayer
- Perfused RBCs treated with:
  - $CaCl_2$
  - Tissue Factor
  - $CaCl_2$ + Tissue Factor
  - $CaCl_2$ + Tissue Factor/Annexin V
- Perfused with 10 dyn of force for 60 minutes or until observed thrombosis



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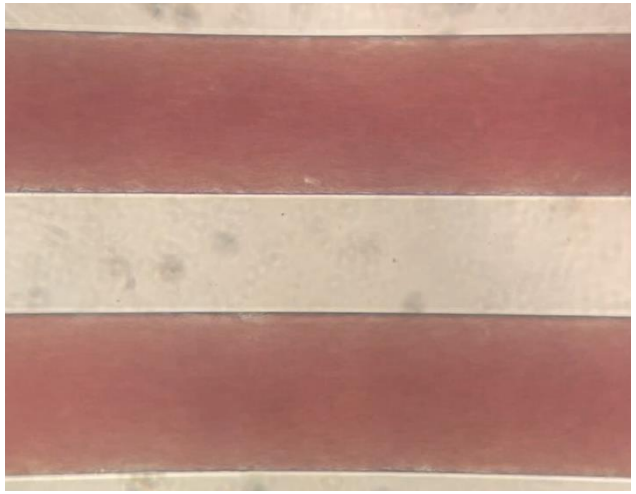
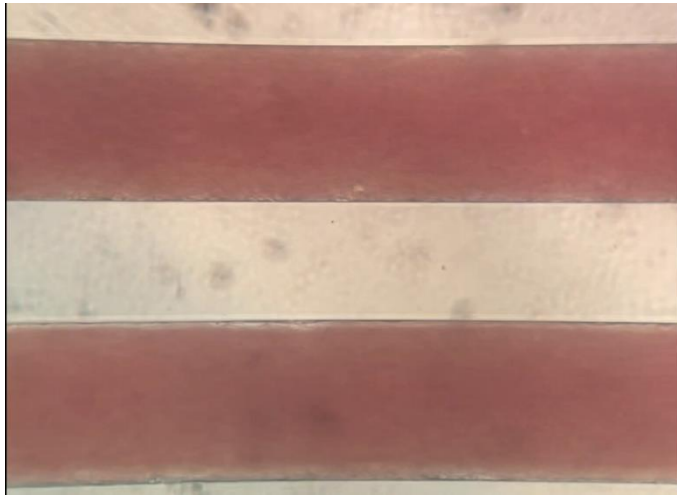
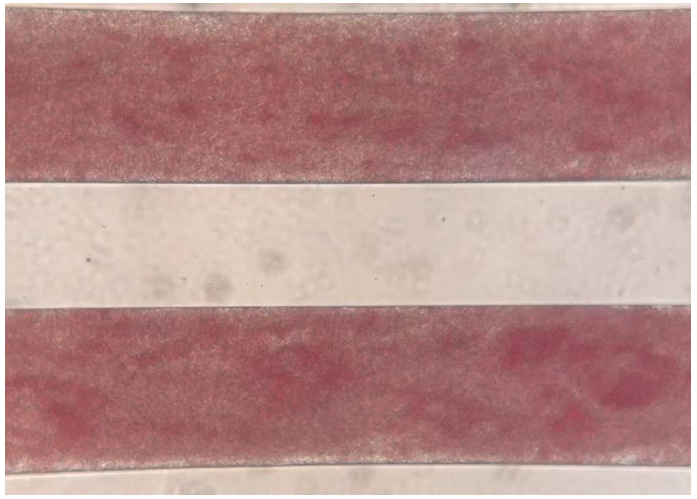
Pulmonary Microcirculatory Perfusion



$CaCl_2$



Tissue Factor



$CaCl_2$ + Tissue Factor

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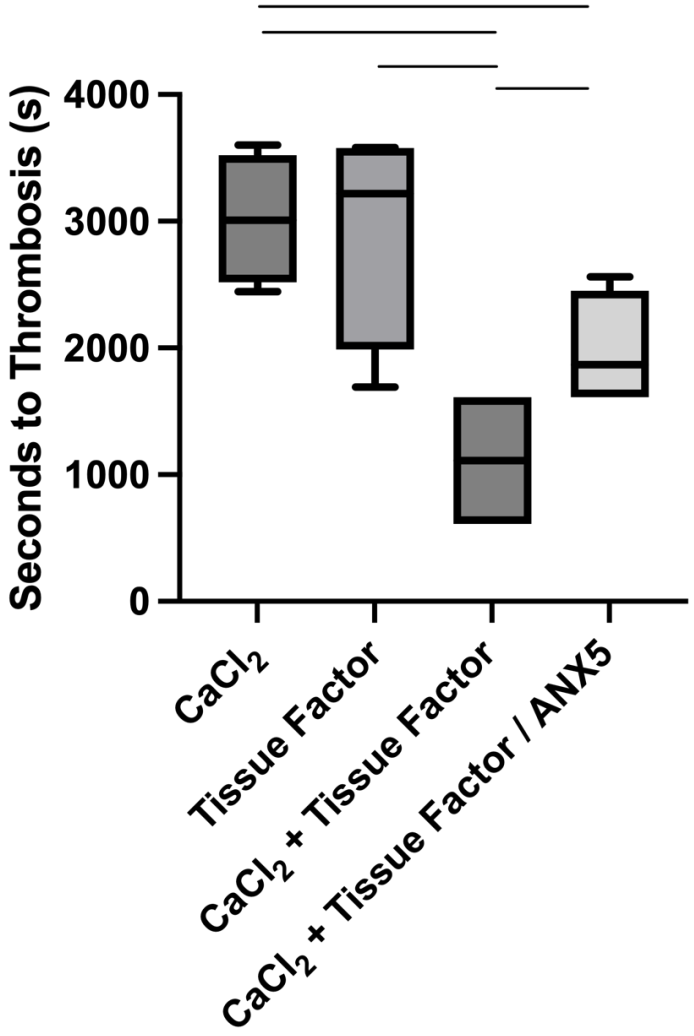
Pulmonary Microcirculatory Perfusion



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- So, RBCs aggregate
- In response to Ca / TF
- Even in flow conditions
- Critical role of PS

- What makes RBC aggregability increase / decrease
  - Concentration
  - Injury pattern

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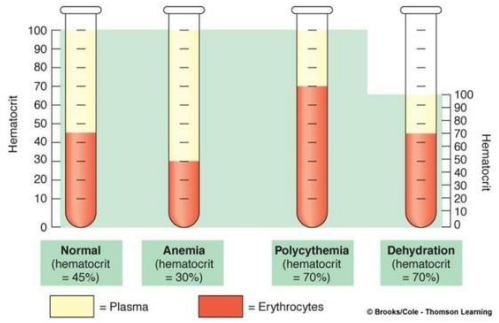
Affecting the RBC Role in Thrombosis



Relative hemodilution is a known contributor to **Trauma Induced Coagulopathy**

Traumatic brain injury increases risk of pathologic thromboembolic disease

Hemorrhagic shock has a multifactorial influence on worsening coagulopathy

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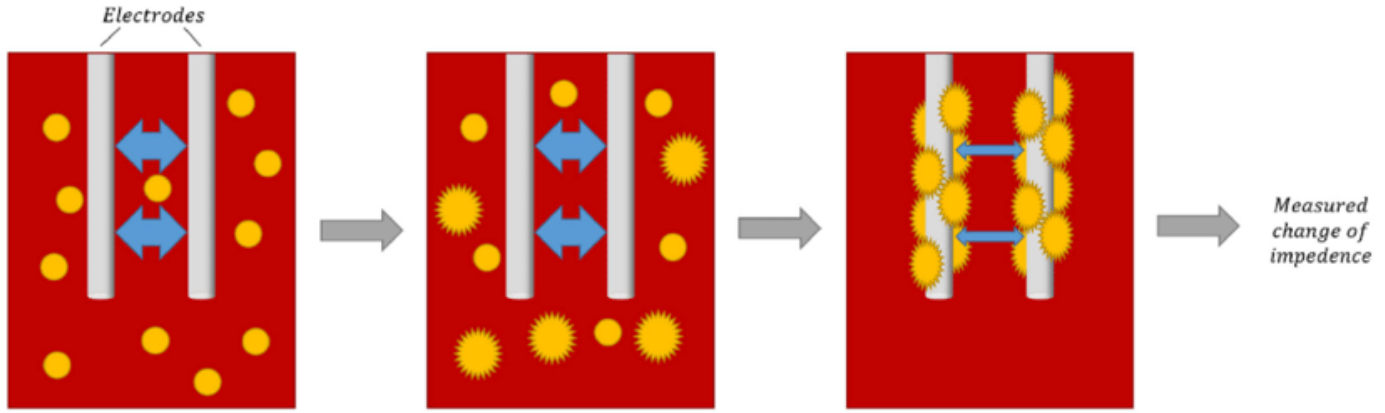
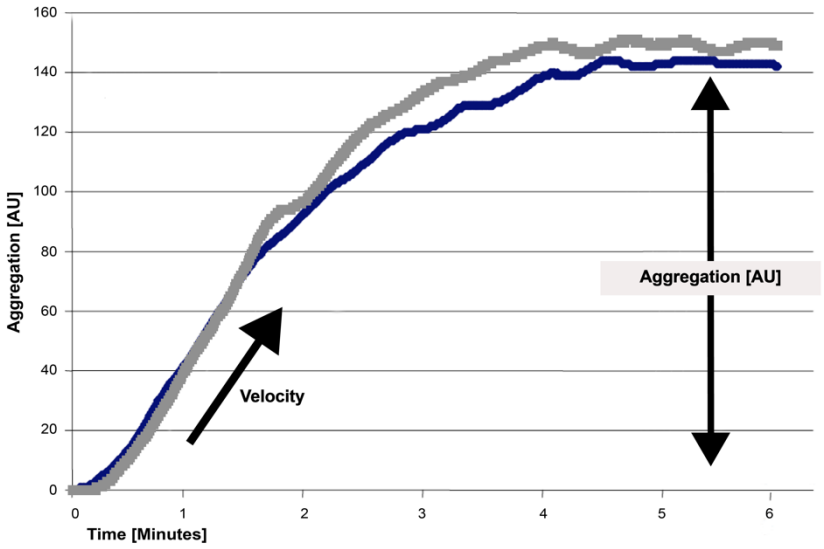
1. Determine the effect of dilute hematocrit (hemodilution) on the aggregation behavior of RBCs

2. Determine the effect of Traumatic Brain Injury and Hemorrhagic Shock on the RBC aggregation and overall contribution to thrombosis

Methods – Impedance Aggregometry





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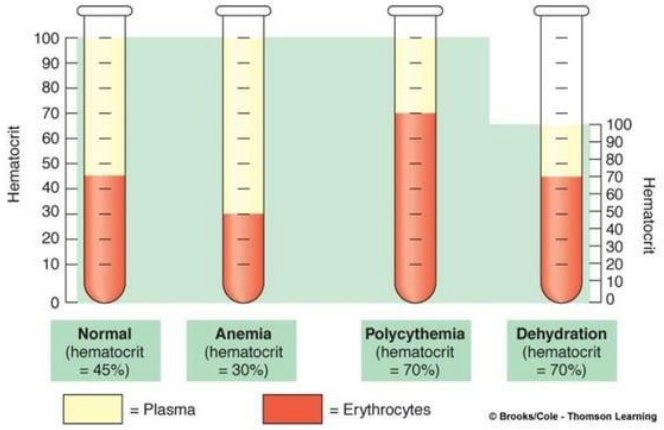
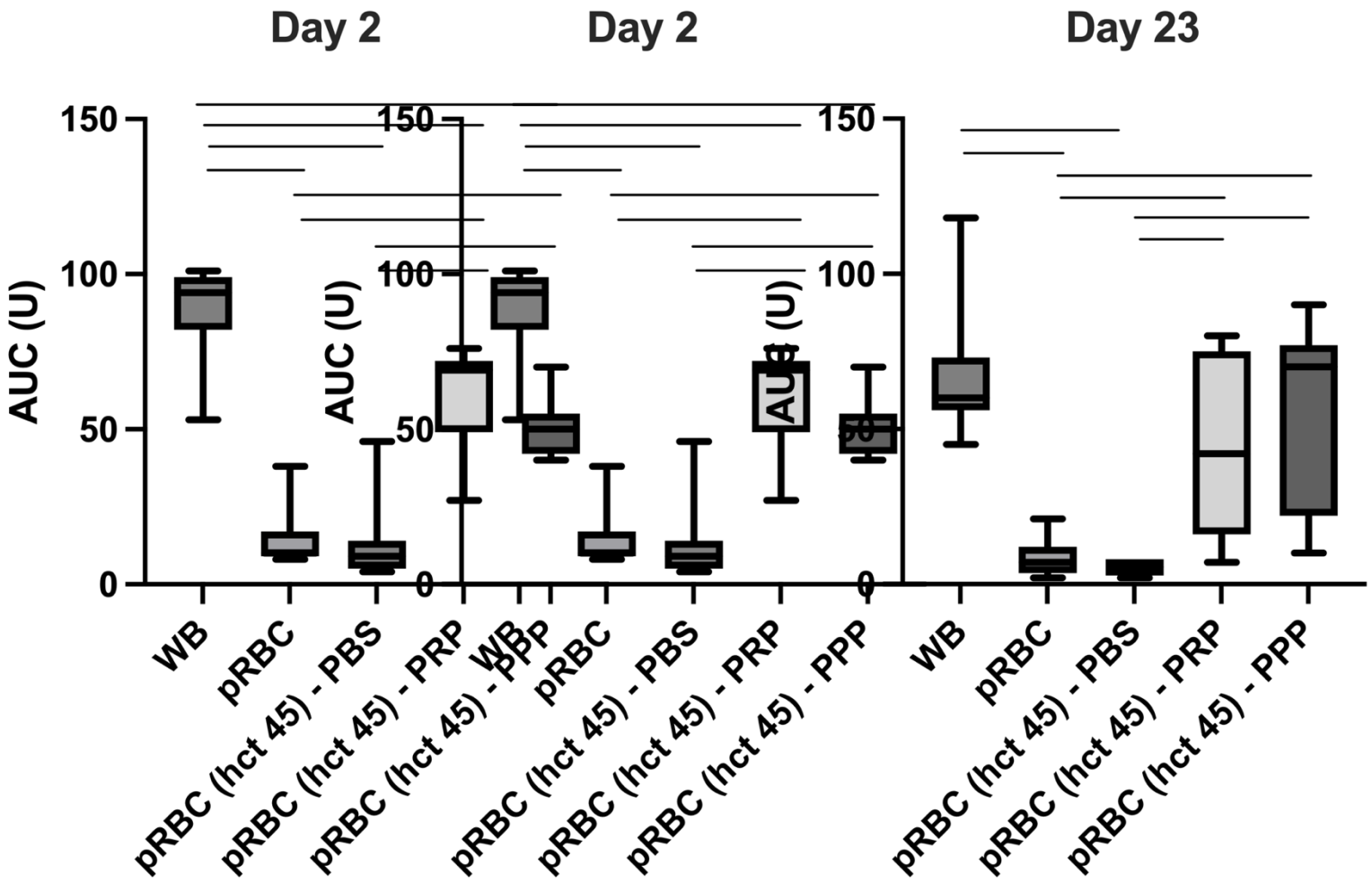
Methods – RBC Dilution

- **Isolated packed Red Blood Cells (pRBC)** in adenine/dextrose solution
  - Coulter cell counter used to determine HCT for each sample (Range ~50-60%)
- For relevant treatment groups, dilution to HCT 45% was achieved and compared to non-dilute samples
- Dilution mediums included:
  - PBS (inert/neutral)
  - PRP (representing reconstituted whole blood)
  - PPP (RBC and plasma with diminished platelet contribution)



RBC Aggregation unchanged with dilution / age



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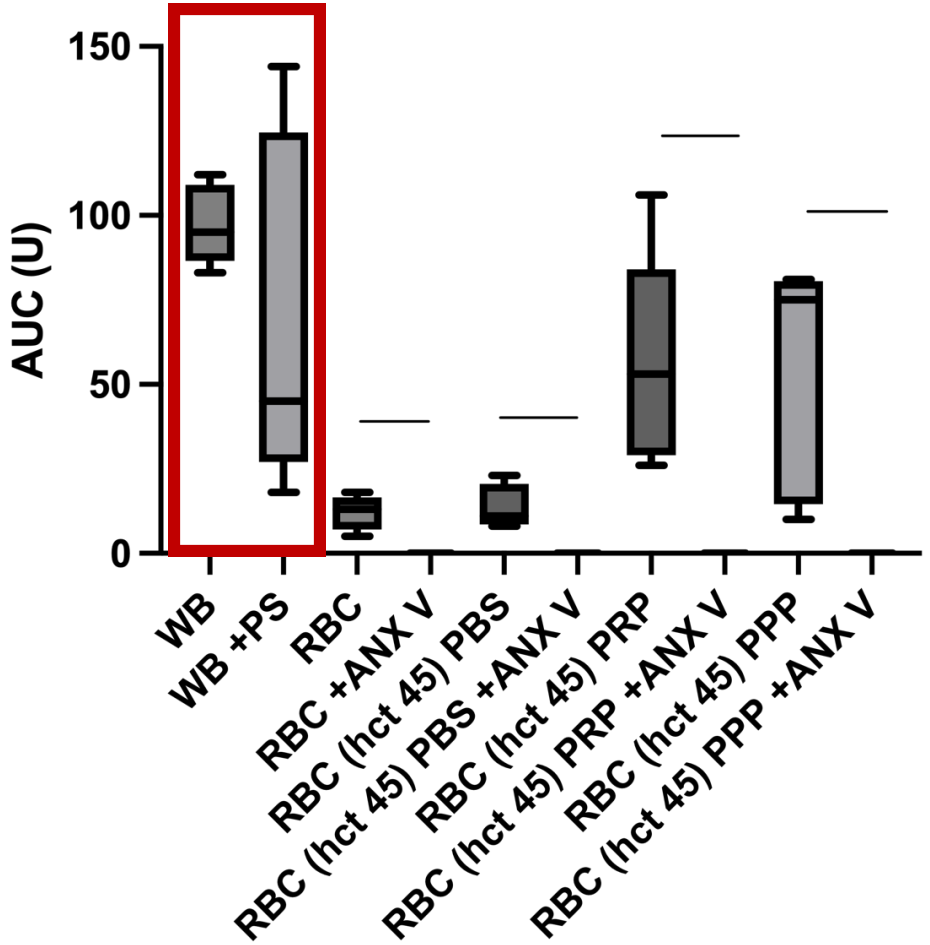
Annexin V blockade still eliminates RBC aggregation?



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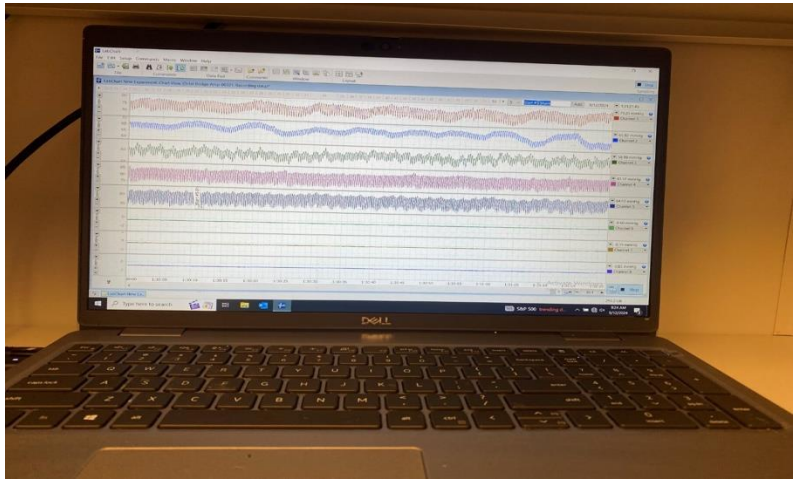
1. Determine the effect of dilute hematocrit (hemodilution) on the aggregation behavior of RBCs

2. **Determine the effect of Traumatic Brain Injury and Hemorrhagic Shock on the RBC aggregation and overall contribution to thrombosis**

Methods – Murine Hemorrhagic Shock

- Monitored hemorrhagic shock induced via controlled bleed
  - Left femoral arterial cannulation
  - MAP of 20-30 mmHg for 60 minutes
  - Resuscitation with Lactated Ringers solution

- Whole Blood Collection/Impedance
  - Completed at 1 hr, 4 hr, 24 hr post-injury
  - Stored in citrate-based storage solution
  - RBCs isolated per 2-spin protocol (previous)
  - Impedance aggregometry post-collection



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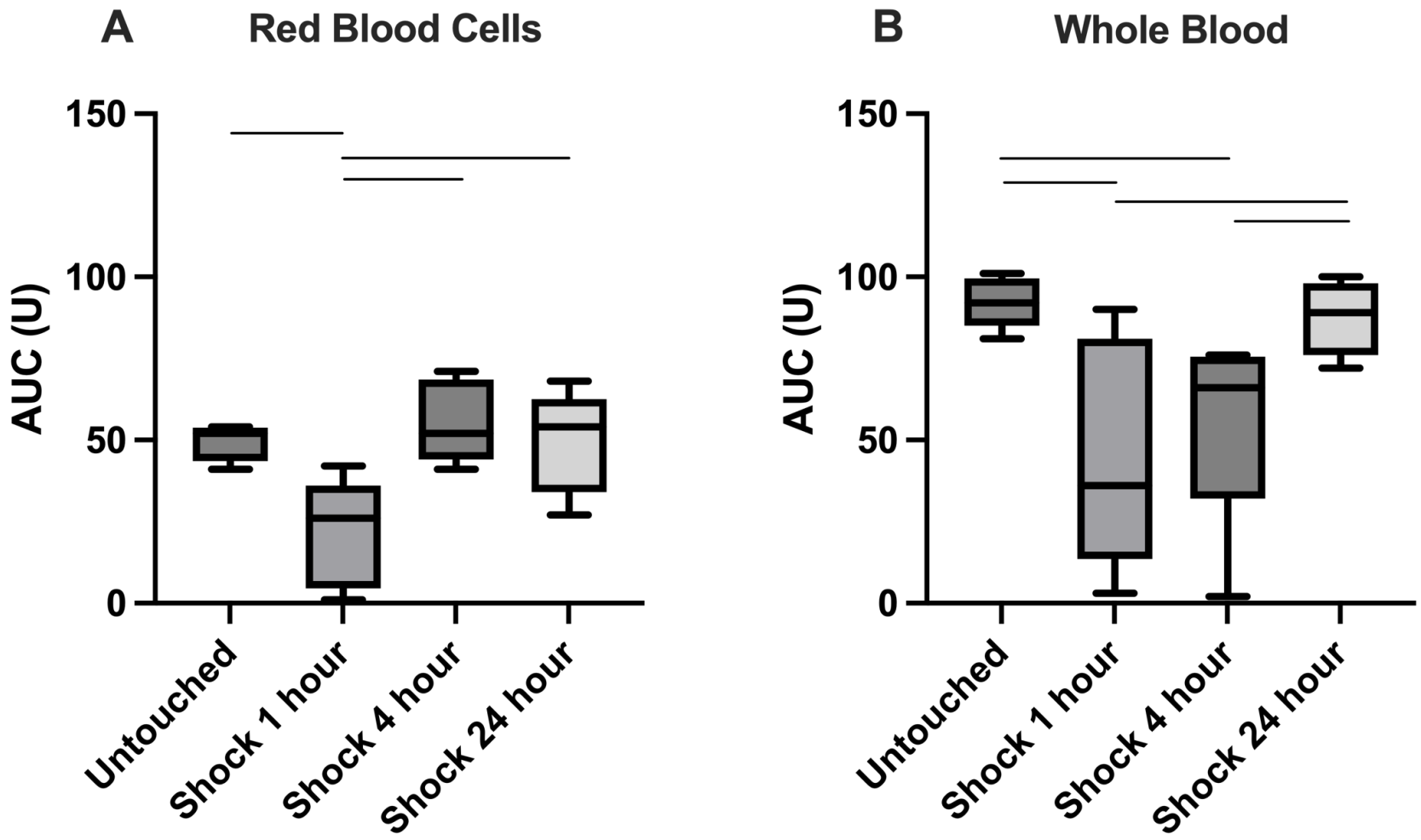
Hemorrhagic shock decreases RBC aggregation



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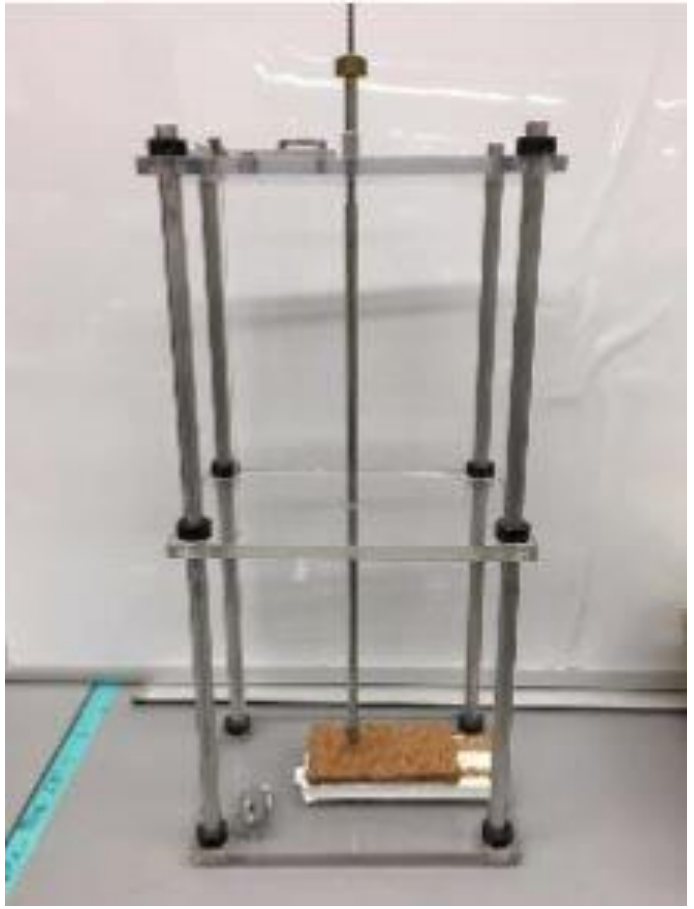
Methods – Murine Traumatic Brain Injury

- Induction of TBI via **Closed Head Injury**
  - Isoflurane anesthetic
  - 400g weight drop from 2cm
- Whole Blood Collection/Impedance
  - Completed at 1 hr, 4 hr, 24 hr post-injury
  - Stored in citrate-based storage solution
  - RBCs isolated per 2-spin protocol (previous)
  - Impedance aggregometry post-collection



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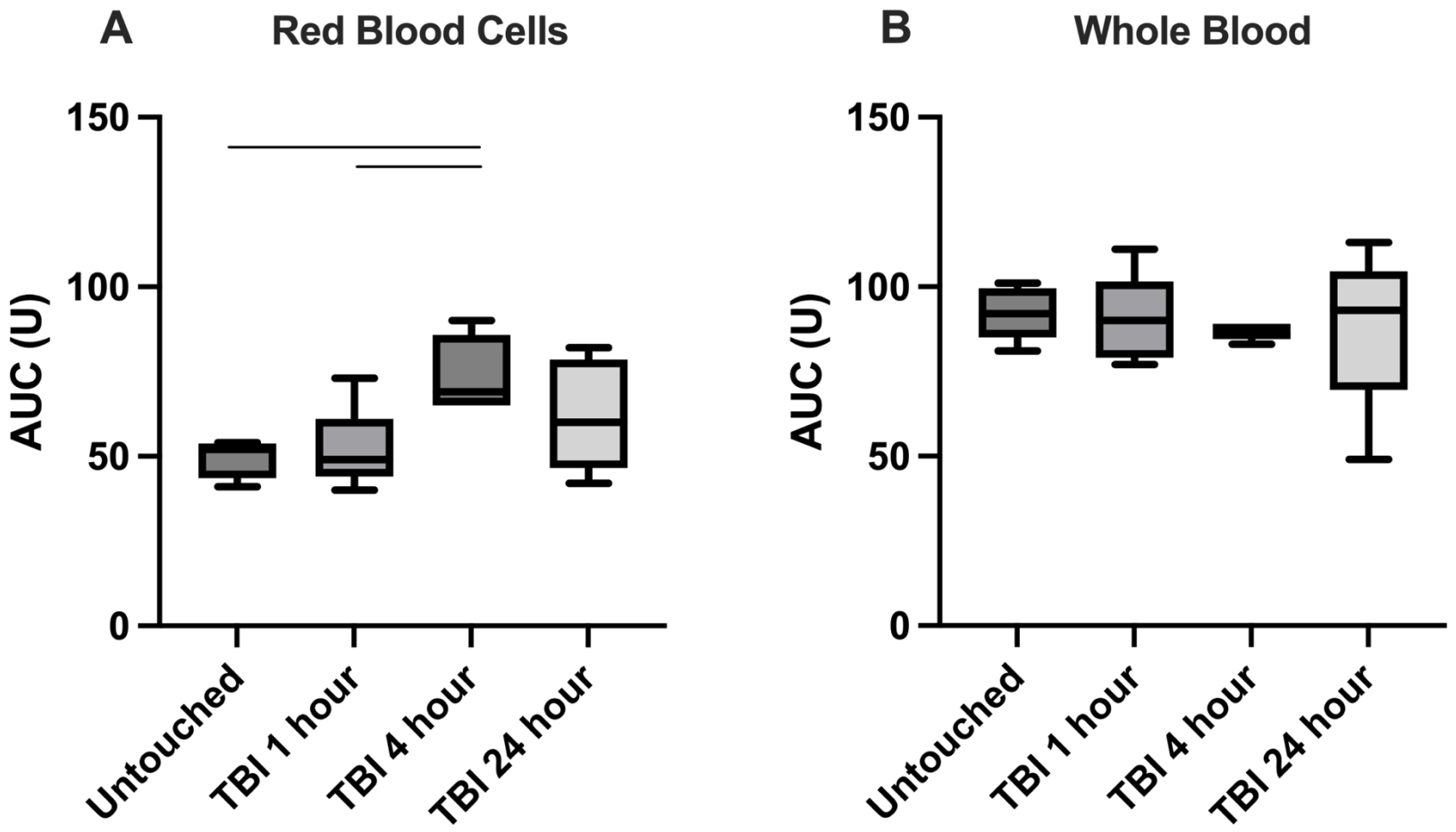
TBI increases early RBC aggregation



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Conclusions

- **Calcium** and **tissue factor** act synergistically to induce RBC aggregation
- **Phosphatidylserine** acts as a key mediator to Ca/TF-based RBC aggregation
- **RBC aging** does not affect aggregation behavior induced by Ca/TF
- RBC aggregation in the presence of transient hypercalcemia and elevated serum TF may act as a nidus for more rapid **thrombus formation** in trauma patients
- In considering thrombosis – don't **Forget About DRE** (Direct Red blood cell Effect)

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Conclusions

- **Mild dilution does not impact aggregation behavior of RBCs** and their potential contribution to thrombus formation
- Clotting factors present in **plasma may influence RBC aggregation potential** more than platelets
- RBC aggregation behavior may **serve a previously unrecognized role in the extremes of hypercoagulability and coagulopathy** in various injury patterns

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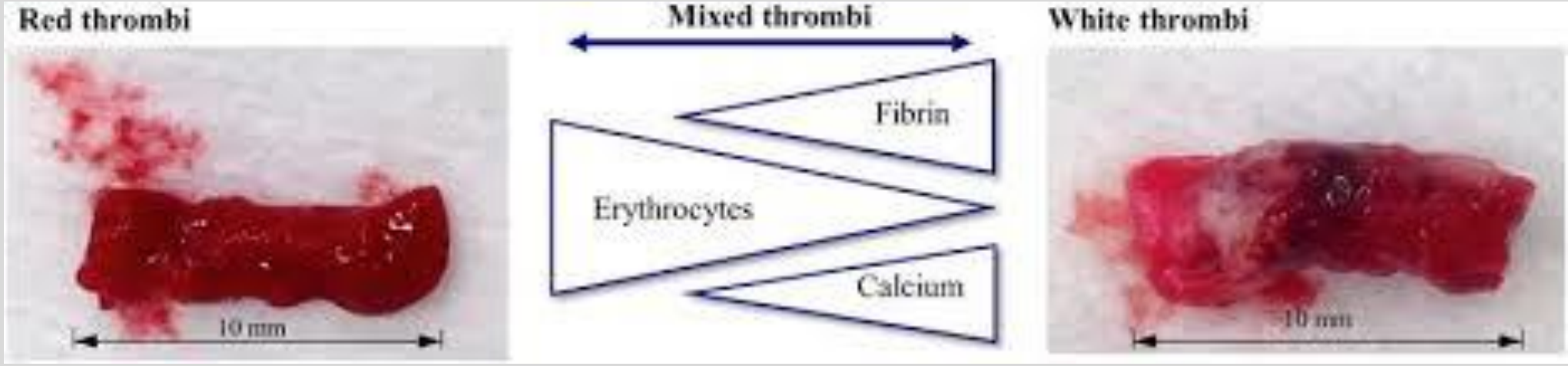
Moving forward

- Characterizing the RBC aggregation – Rouleaux? Agglutination?
- Studying phosphatidylserine mobility
- Are clots created equal and what is the RBC role after trauma?



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