Coagulation Changes Related to RBC Transfusion

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Virchow's Triad Revisited

The roles of RBCs are commonly neglected

Early Indirect Evidence for the Role of RBCs in Hemostasis & Thrombosis

- Anemic and thrombocytopenic patients with bleeding can be successfully treated by elevating the hematocrit.
- Abnormally high hematocrit can predispose a patient to thrombotic disease (polycythemia vera).
- Inherited red blood cell abnormalities can predispose to premature thrombosis (sickle cell disease, β-thalassemia, hemolytic anemias, stomatocytosis).
- Diseases that secondarily alter the properties of red blood cells can result in thrombosis (diabetes, hypertension, leg vein thrombosis, coronary heart disease).
Blood Flow at High and Low Shear Rates:
Margination of Platelets by RBCs

Red Blood Cell Phosphatidylserine Exposure Contributes to Thrombin Generation

0.6% of RBCs express phosphatidylserine, supporting prothrombinase, also minothrombinase, as an anticoagulant.

In healthy RBCs, membrane asymmetry is maintained.

With RBC damage or certain diseases, phosphatidylserine exposure can increase.

Red Cell Microparticles
Increase with disease & storage; procoagulant
Direct Interactions of RBCs and Platelets Under Venous Shear

Fibrinogen Binds to an Integrin on RBCs and May be Involved in RBC-RBC interactions

Clot Contraction (Retraction)

The volume shrinkage of clot that is driven by platelets and fibrin(ogen)
Significance of Clot Contraction

Hemostasis

Restoring blood flow past obstructive thrombi

Wound healing
Molecular Mechanism of Cellular Contraction

Contraction is initiated by signaling processes when platelets are activated. Force is generated by non-muscle myosin IIa pulling on actin filaments inside platelets, transmitting the force to fibrin outside the cell via the integrin αIIbβ3.

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Figure 19-55 Molecular Biology of the Cell 6e (© Garland Science 2015)

Contracted Whole Blood Clots by Scanning Electron Microscopy

Inside polyhedral erythrocytes = polyhedrocytes


Outside fibrin & platelets
Major Observations

• In contracted clots, platelets and fibrin are primarily on the surface, while erythrocytes are mainly on the interior.

• In contracted clots, erythrocytes are compressed to close-packed polyhedral structures.

  Polyhedral erythrocytes  →  Polyhedrocytes

• Polyhedrocytes form a nearly impermeable seal to stem bleeding and help prevent vascular obstruction, but confer resistance to thrombolysis.
Polyhedrocytes are present in unclotted blood centrifuged at 1000g or greater: minimal stress required is 75-150 dyne/cm²

Kinetics of Clot Contraction Studied by Measuring Volume Change over Time

Diagram A: Diagram showing the setup of a CCD camera, source of light, cuvette, and blood clot in water at 37°C.

Diagram B: Images showing clot size at 0 min, 10 min, and 20 min.

Diagram C: Graph showing relative clot size (%) over time (seconds) with data points at 0, 1, 2, and 3.
RBCs Lessen the Extent of Contraction

**Graph:**
- **X-axis:** Time (seconds)
- **Y-axis:** Relative Clot Size (%)
- **Lines:**
  - Red line: With RBCs
  - Blue line: Without RBCs

**Bar Chart:**
- **Groups:**
  - Without RBCs
  - With RBCs
- **Y-axis:** Extent of Contraction (%)
- **Comparison:** The extent of contraction is significantly lower with RBCs compared to without RBCs, indicated by the *** symbol.
Naturally Stiff RBCs Have a Reduced Rate and Extent of Contraction

- Llama RBCs: Ovalocytes, 2x Spectrin, Much Stiffer
Sickle Cell Disease Patients Have Reduced Clot Contraction

- **Graph:**
  - X-axis: Time (sec)
  - Y-axis: Relative Clot Size (%)
  - Line graphs for Control and Sickle Cell Disease Patients

- **Bar Chart:**
  - X-axis: Control, Sickle Cell Disease Patients
  - Y-axis: Extent of Contraction (%)
  - Comparison between Control and Sickle Cell Disease Patients
  - Statistical significance indicated by ****
RBCs Increase the Generation of Contractile Force

**Reconstituted Blood**

**Whole Blood vs PRP**

![Graphs showing the effect of RBCs on contractile force](image)

- **Without RBCs**
- **With RBCs**

Contractile Stress (Pa)

Time (sec)

Contractile Stress/platelet (Pa)

Time (sec)

n>3

* p<0.05
** p<0.01
*** p<0.001
Formation and composition of arterial and venous thrombi

Mouse saphenous vein clots contain polyhedrocytes, platelets, and fibrin.

Polyhedrocytes make up 42% of the clots.
Coronary Artery Thrombus

1. Aorta
2. Right Coronary Artery
3. Left Anterior Descending Coronary Artery
4. Circumflex Coronary Artery
5. Left Main Coronary Artery
Overall Composition of Human Coronary Artery Thrombi
RBCs Are More Prevalent than Expected

Polyhedrocytes Are Present *in vivo* in Coronary Artery Thrombi


Pulmonary Emboli and Venous Thrombi Contain a Large Proportion of Polyhedralocytes
Extent and rate of clot contraction are reduced in the blood of ischemic stroke patients.
Conclusions on RBC Involvement in Hemostasis and Thrombosis

• In flow, RBCs cause margination of platelets.
• RBC phosphatidylserine is responsible for significant prothrombin generation.
• RBCs bind to platelets and to each other.
• In contracted clots, erythrocytes are compressed to close-packed polyhedrocytes.
• Polyhedrocytes are present in thrombi.
• Closely packed polyhedrocytes in contracted clots are important for hemostasis.
• Polyhedrocytes confer resistance to fibrinolysis by decreasing permeability.
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