RED BLOOD CELL STORAGE

as gleaned through integrated omics

Angelo D'Alessandro, PhD
Director, Metabolomics Core
Department of Biochemistry and Molecular Genetics
University of Colorado Denver – School of Medicine
DISCLOSURE

• Co-Founder of Omix Technologies inc;
• SAB member for New Health Sciences inc

FUNDING

PI
• Linda Crnic Institute – Trisome project on Down Syndrome; (PI)
• National Blood Foundation – 2016 Grant Cycle (PI)

Metabolomics Core
• School of Medicine – UC Denver – Dean’s CORE START UP funds;

Co-investigator
• P50 – Trauma Activates Cells – NGMS; (PI: Banerjee)
• RO1 – Metabolic reprogramming of RBCs in Sepsis; (PI: Doctor A)
• DOD – Metabolic reprogramming in pulmonary hypertension; (PI: Stenmark K)
• DOD – Metabolomics of triple negative breast cancer; (PI: Richer J)
RBC storage lesion: well established area of investigation

1. GLUT
2. LAC
3. Hb
4. Ca/K pump
5. Glycolysis
6. Pentose Phosphate Pathway
7. Glycation
8. Mitochondria
9. Microvesicles
10. Micr vesicles (180nm)

CD47
Rh
Hb
PLA2
CPA

ROS

CD17

Glycosylation of membrane proteins

NADPH

GSH

GSSG

Protein oxidation

Band 3 Clustering

PS exposure in the outer leaflet

Lipid peroxidation

Proteasome

Smases

Actin Spectrin

AE1

HCO3

Proteomics (PRE, AIP, PK)

Caspases (Proteases)

D'Alessandro et al. Transfusion 2016; Zolla et al. Blood Transfusion 2015;
CONFERECE REPORT

2015 proceedings of the National Heart, Lung, and Blood Institute's State of the Science in Transfusion Medicine symposium

Steven L. Spitalnik,1 Darrell Triulzi,2 Dana V. Devine,3 Walter H. Dzik,4 Anne E. Eder,5 Terry Gernsheimer,6 Cassandra D. Josephson,7 Daryl J. Kor,8 Naomi L. C. Luban,9 Nareg H. Roubinian,10 Traci Mondoro,11 Lisbeth A. Welniak,11 Shimian Zou,11 and Simone Glynn,11 for the State of the Science in Transfusion Medicine Working Groups*

4. “How can we make better products?” Develop improved methods for preparing classical products and determine whether there are alternative ways of preparing analogues (e.g., synthetic, bioengineered, “biopharmaced”).

BLOOD BANK STORAGE QUALITY

We can use Omics to make it better
RBC storage: how long is too long?

AS3: Significant metabolic lesions tend to accumulate by storage day 14

Metabolites increasing by storage day 14
Metabolites decreasing within storage day 14

RBC storage: ...and GAPDH oxidation, affecting activity and potentially band 3 binding

![Diagram of glucose metabolism and lactate production](image)

- **Glucose** → **GAPDH** → **GDP** → **Lactate**
- **GLUT** and **MCT** transport glucose and lactate

Graph showing changes in ATP, DPG, Lactate, and Glucose over storage days:

- **ATP** decreases over time
- **DPG** increases over time
- **Lactate** increases over time
- **Glucose** decreases over time

Graph showing oxygen saturation (% saturation) vs. oxygen hemoglobins (% saturation) vs. PO2 (mmHg):

- **Left shift**: Decreased temp, Decreased 2-3 DPG, Decreased [H+], CO
- **Right shift**: Increased temp, Increased 2-3 DPG, Increased [H+]

[Source: Haines et al. Blood 2016; under review]
From energy to redox: stored RBCs are challenged with oxidative stress.

Oxygen jumps from one Hb molecule to another, a process that promotes formation of oxygen radicals and triggers Haber Weiss and Fenton reactions.

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Winterbourne et al. 1978; DAlessandro et al. 2015; Spitalnik and Francis, 2015
Oxidative stress targets: ROS target the most abundant RBC proteins

Hemoglobin Beta (HBB)

M1 H3 H64 H93 C94 H144

Oxidation

Deoxygenated Hemoglobin

Iron

Oxidized proximal Histidine of HBB

Oxygen

Iron

Oxygenated Hemoglobin

Wither et al. Transfusion 2016 – Cover of the February 2016 issue
RBC antioxidant capacity: GSH synthesis is compromised during RBC storage.
GAPDH: redox sensitive thiols at the interface between energy and redox metabolism

![Diagram showing the GAPDH activity over days 2, 21, and 42.](image)

- **Day 2**: High GAPDH activity.
- **Day 21**: Moderate GAPDH activity.
- **Day 42**: Low GAPDH activity.

**Diagram Notes:***
- GLUCOSE
- GAPDH
- G3P → DPG
- Oxidative stress
- Pentose Phosphate Pathway
- NADPH

**References:**
- Castagnoli et al. Blood Transfusion 2010
- Rogers et al. 2012
- Low et al. 1992
RBC storage: steady state of PPP are depressed, not fluxes!

- **13C\(_{1,3}\)-Glucose**
- **13C-Lactate**
- **13C\(_3\)**
- **13C\(_2\)+13CO\(_2\)**

Graph:
- PPP
- Glycolysis

Time (days):
- 42 days
- 0, 7, 14, 21, 28, 35, 42

Haines et al. Blood 2016; minor revision
RBC supernatant: markers of STORAGE QUALITY

HBA1
Y = 206.62 X + 970.29
R^2 = 0.974

HBB
Y = 2172.2 X + 1742.89
R^2 = 0.972

Day 42 top proteins by abundance – pmol/ug

r^2 = 0.972
r^2 = 0.974

GAPDH pmol/ug

HBA1
HBB

r^2 = 0.982

D'Alessandro et al. Transfusion 2016; June 2016 issue cover
RBC storage: Vesiculation impairs RBC morphology and promotes osmotic fragility

Haemolysis curve

Hemolysis at Lower than Physiological [NaCl]

- day 0
- day 7
- day 14
- day 21
- day 28
- day 35
- day 42

Haemolysis (%) vs. NaCl (%)
02 RBC anaerobic storage
RBC storage: Potential benefits of hypoxia

- **Normoxia**
  - Glucose metabolism
  - Alkalization
  - Band 3
  - Bohn effect

- **Hypoxia**
  - Oxidative stress
  - pH Alkalization
  - Reduced activity

Processes include:
- Glucose
- Oxidative stress
- ATP
- NADPH
- Lactate

**Key Pathways**:
- Glycolysis
- Pentose Phosphate Pathway
- Alkalization Pathway

**Marked Changes**:
- Decrease in oxidative stress
- Increase in pH alkalization

**Figures**:
- **Fig. 1**: Overview of RBC storage with key processes.
- **Fig. 2**: Detailed metabolic pathways under normoxia and hypoxia conditions.

*(D'Alessandro et al. ISBT 2016)*
RBC storage: Hyperoxia promotes the mechanisms

- **Hyperoxia**
- **Control**
- **Hypoxia**

Red Blood Cell Unit

$^{13}C_{1,2,3}$-GLUCOSE

$\textbf{Oxygen Saturation (SO}_2\textbf{)}$

- **Normoxic**
- **Hyperoxic**
- **Hypoxic**

**Storage Days**

**Methemoglobin**

**Storage Day**

$n = 4$ per group

Haines et al., Blood 2016, under review
Anaerobic storage: Improved energy metabolism

Graphs showing changes in DPG and ATP levels over storage days under hypoxia, control, and hyperoxia conditions.

Cassagnola et al. Blood Transfusion 2010; Rogers et al. 2012; Low et al. 1992
Anaerobic storage: Improved energy metabolism
Anaerobic storage: residual PPP activation + hypoxia keep in check oxidative stress

GSH/GSSG ratios (A.U.)

Control
Hyperoxia
Hypoxia

D'Alessandro et al. in preparation
Anaerobic storage: preserves RBC morphology, hemolysis and reduces osmotic fragility.
RBC storage – Future directions: How can we design a better storage strategy?

- Anaerobic storage
- Antioxidants (Vitamin C + NAC)
- Rejuvenation
- Cryopreservation
- Alkaline additives
- Anaerobic storage + CO₂
- Filtration/Washing
- G6PDH deficiency
THANK YOU for your attention

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