Assessment of Blood Trauma in Circulatory-assist Devices

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Multi-scale Thrombosis Modeling Seminar Series
Experimental subgroup meeting

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Motivation for study

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Mechanical blood trauma

• Major concern in the development of circulatory-assist devices

• Mechanical stresses cause [Kameneva and Antaki, 2007]:
  - overstretching or fragmentation of subpopulation of red blood cells (RBCs) causing hemolysis
  - activation or dysfunction of platelets and leukocytes
  - increased concentrations of inflammatory mediators
  - sublethal blood trauma
Sublethal blood trauma

- Sublethal blood trauma [Hung et al. 1989, Kameneva et al. 1995]:
  - increase in RBC aggregability
  - increase in RBC mechanical fragility
  - decrease in RBC deformability
  - increase in blood viscosity

- Sublethal RBC mechanical damage is known to:
  - decrease RBC life span due to removal by the spleen [Sandza et al. 1974]
  - decrease the density of functioning capillaries
  - decrease the contact surface area of RBCs with capillary walls
RBC Aggregation

• RBC aggregation depends on concentration of plasma fibrinogen and some globulins, cell surface charge and deformability

• Increased aggregability is associated with inflammatory reaction, etc., or with damage to RBCs which reduces cell surface charge

Human blood viewed through a light microscope. RBC aggregates are mostly due to bridging of cells with fibrinogen molecules.

Photo by M. Kameneva
Decreased RBC deformability

- Due to deformability, 8 µm human RBCs are able to enter and pass 3-5 µm capillaries
- Blood viscosity and elasticity significantly depends upon RBC deformability and can be used to detect sublethal blood trauma

RBC passing through a narrow slit in spleen
http://www.redcross.org/services/biomed/research/spleen.html

RBC passing through a filter pore
http://www.mechatronics.nl/products/lorrca/body.html
Newtonian vs. Non-Newtonian fluids

• **Newtonian fluid**: Viscosity of fluid is independent of shear rate

• **Non-Newtonian fluid**: Viscosity of fluid is dependent of shear rate
  - Shear-thinning fluid (blood)
  - Shear-thickening fluid (corn starch suspensions)

![Graph showing viscosity of human blood and artificial blood versus shear rate](image-url)
Blood viscoelasticity

Under oscillatory flow, blood exhibits both viscous (energy dissipative) and elastic (energy storing) characteristics

**Figure:** Comparison of viscosity [A] and elasticity [B] of bovine, ovine, and porcine whole blood samples at 30% Ht at 40 to 220 s\(^{-1}\) shear rates

Marascalco et al. ASAIO J 2006.
Measurement of blood viscoelasticity

Measurements performed in a Vilastic-3 Viscoelasticity analyzer

- Oscillatory flow in a stainless steel capillary tube (0.051 cm inner radius, 6.18 cm length)
- Oscillation frequency: 2 Hz
- Shear rate: 20 to 250 (s$^{-1}$)
- Constant temperature: 25°C
Assessment of RBC deformability

**Blood Viscoelasticity**

- Donor RBC viscosity and elasticity increased during seven weeks of storage
- RBC mechanical fragility increased during storage time
Micro Assessment of RBC deformability

Linkam CSS450 Optical Shearing System
Method: Linkam optical shearing system

- Samples: whole blood and 7% polyvinylpyrillidone (PVP) with ~30 cP viscosity
- Final concentration: 0.5% Ht in ~1 ml PVP
- Shear rate range: 100-1000 s^{-1}
- 100 images of RBC deformation recorded at each shear rate studied

Schematic of the shearing stage showing the sample location and microscope imaging lens

Figures by R. Zhao
Method (cont): Image processing

- Image analysis via NIH ImageJ software: ellipse-fitting measurement
- Processed images yield an RBC elongation index (EI) that characterizes cell deformability

![Image of cell analysis software](image.png)

\[ EI = \frac{a - b}{a + b} \]

- \( a \) = major ellipse axis
- \( b \) = minor ellipse axis
My RBCs in Linkam device

Human RBCs at 100 s\(^{-1}\) shear rate 
(El=0.10)

Human RBCs at 1000 s\(^{-1}\) shear rate 
(El=0.34)
Effect of exposure time on the threshold shear stress (Leverett et al., 1972)

- Exposure time (sec):
  - 1E-6
  - 1E-4
  - 1E-2
  - 1E+0
  - 1E+2
  - 1E+4

- Shear stress (Pa):
  - 1E+0
  - 1E+2
  - 1E+4

Figure by M. Kameneva

- Safe
- Hemolysis
- Sublethal trauma

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Literature values of shear stresses and exposure times reported for sublethal blood damage

<table>
<thead>
<tr>
<th>Shear stress (Pa)</th>
<th>Exposure time (s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4000</td>
<td>0.00001</td>
<td>Blackshear, 1972</td>
</tr>
<tr>
<td>300</td>
<td>0.4</td>
<td>Paul et al., 2003</td>
</tr>
<tr>
<td>200</td>
<td>0.6</td>
<td>Paul et al., 2003</td>
</tr>
<tr>
<td>120</td>
<td>15</td>
<td>Baskurt et al., 2004</td>
</tr>
<tr>
<td>56</td>
<td>180</td>
<td>Lee et al., 2004</td>
</tr>
</tbody>
</table>

Shearing system to study hemolysis at given shear stresses and exposure times (Ongoing)

Pressure transducer (Abbott Laboratories)

Stainless steel pipe (955 μm ID, 12” length)

Pressure transducer (Abbott Laboratories)

\[ \Delta P = \frac{8\mu LQ}{\pi r^4} \]

PHD2000 syringe pump (Harvard Apparatus)
Methods

• Washed bovine RBC suspensions in PBS (Ht = 40%)
• Performed test conditions: 20-50 ml/min flow rate, 19-33 Pa wall shear stress, 1-7 sec exposure time
• Hemolysis (plasma free hemoglobin, plfHb) measured before and after exposure to prescribed shear stresses for given times
• RBC deformability measured in the same samples using the Linkam system
Preliminary results

Hemolysis at tested wall shear stresses and exposure times
Effect of exposure time on the threshold shear stress (Leverett et al., 1972)

Exposure time (sec) vs. Shear stress (Pa)

- Hemolysis
- Safe

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Further studies using our Dual-syringe system

• Study shear flow of platelet-rich plasma (PRP) and RBCs
• Concerns about surface effects on platelet activation

Potential solutions

- Use a surface coating to prevent platelet adhesion (e.g. MPC developed by Dr. Ye in Professor Wagner’s laboratory)
- Use a capillary tube made of a different material (glass) coated with albumin
Effect of shear on ADP release in whole blood, suspension of RBC in PFP and PRP with RBC ghosts

Alkhamis et al. ASAIO Trans 34 (1988)
Proposal for future experiments

Effect of shear on platelet activation with and without RBCs

Can be tested: PDMS 200 µm or 50 µm ID Y-channel

Buffer or RBC suspension
Other relevant mechanical blood trauma projects
Drag-reducing Polymer Solution as a Test Fluid for In vitro Evaluation of Blood Damage in Blood Pumps

Presented at BMES 2009 conference and Published ASAIO Journal 56 2010, 6-11

Amanda R. Daly, Hideo Sobajima, Salim E. Olia, Setsuo Takatani, Marina V. Kameneva

University of Pittsburgh, McGowan Institute for Regenerative Medicine, Pittsburgh, PA, United States
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan
• Study objective: Use DRP for in vitro testing of potential blood damage produced by circulatory-assist devices

• Main components of flow system: glass capillary (induce turbulent flow), Levitronix CentriMag or Bio-Pump BPX-80 pumps

• Characterize DRP degradation and hemolysis: record flow rate decrease at constant pressure and measure plfHb in samples collected during 2 hour tests

• Conclusion: DRP mechanical degradation in a flow system with a blood pump may provide a sensitive, standard method for the evaluation of potential pump hemolysis without the use of blood
Circulating blood volume affects the results of in vitro hemolysis tests of blood pumps

Presented at ASAIO 2009 conference

Amanda R. Daly, Salim E. Olia, Timothy M. Maul, Marina V. Kameneva

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In Vivo Hemorheological Assessment of Biocompatibility of PediaFlow™ VAD: Comparison with Sham Implants

Presented at BMES 2009 conference

Amanda Daly, Salim Olia, Shawn Bengston, Timothy Maul, Peter Wearden, Harvey Borovetz, James Antaki, Marina Kameneva

Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh;
Children’s Hospital of Pittsburgh, University of Pittsburgh Medical Center;
Department of Biomedical Engineering, Carnegie Mellon University;
Pittsburgh, Pennsylvania, USA
• **Study objective:** Evaluate and compare potential blood trauma in sham, PediaFlow Gen 2 (PF2) and Gen 3 (PF3) implanted sheep

• **Hemorheological evaluation:** Blood samples collected for plfHb, hematocrit, plasma protein and fibrinogen concentrations, whole blood and plasma viscosity measurements

• **Conclusions:** Mean values of plfHb were similar in sham and implanted sheep

• General trends in Ht, fibrinogen and total plasma protein concentrations were very similar

• PediaFlow™ VAD demonstrated excellent biocompatibility in an ovine model
FDA Critical Path Project
Blood Damage in Medical Devices (ongoing study)

Motivation for study

• Standardize hemolysis testing of investigational blood pumps using a standard flow loop, nozzle and test conditions
• CFD analysis performed by 50+ groups to evaluate hemolysis produced by the nozzle model
• FDA, Cleveland Clinic and Kameneva laboratory performing experiments to validate computational analysis of blood damage produced by nozzle model
FDA blood damage study flow loop

Blood bag (500 mL) | Reusable reservoir

P_out and sample port | Temperature

Nozzle model w/removable inlet and outlet steel tubing

PVC tubing (½” ID, 11/16” OD)

Stainless Steel Tube (0.39” ID, 7/16” OD)

Water bath/heat exchanger

Flow probe

Fill and drain port

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FDA blood damage nozzle model

Model dimensions:

- Throat: 0.04 m
- Diameter: d = 0.004 m
- Length: 0.012 m
- Angle: 20°
Methods

- Bovine blood (Ht = 36%)
- Test time: 120 minutes
- Blood volume: 300 ml
- Flow Rate: 6 LPM
- Model condition: nozzle in sudden contraction or cone direction
- Control condition: nozzle replaced with ¼” tubing; throttle used to produce equivalent $\Delta P$ as the nozzle
- Plasma free hemoglobin measured for all samples
Acknowledgement

NIH R01 Multiscale Model of Thrombosis
Thank you!
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