Possibilities of Thrombogenicity testing by *In Vitro* Systems

Wim van Oeveren
Haemoscan
Groningen
The Netherlands
Topics to be addressed

- Pro's and Con's of in vitro models
- General overview of various available in vitro methods
- Flow loop model specifics
- In vitro assay validation
- Device geometry
- Investigate material changes in a marketed device
Advantage small in vitro system

- Volume from 3,5 ml
- 1 Volunteer for all test and reference samples
- Reproducible per donor
- Puls, Flow and shear adjustable
- Low costs
- All immuno assays are commercially available (anti-human antibodies)
- Results of a complete study in short time
- Small materials for testing (from 0,09 cm2)
Limitations small in vitro system

- Long duration of testing not possible
- Effects endothelial cells ignored
- No feedback functions (from organs)
- No surgical effects (incl release factors)
- Anatomy differences
- Aspecific blood activation (drawing, circuit)
- Anticoagulant needed
- Effects exagerated (ratio device/blood and accumulation)
Blood circulation models

- Chandler
  - Air
  - Luer lock

- Hemobile
  - Ball valve

- Roller pump
  - Waterbath

Blood compatibility and biomarker detection
Flow loop model specifics:

++ = good

<table>
<thead>
<tr>
<th></th>
<th>Chandler</th>
<th>Pump</th>
<th>Hemobile</th>
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<tr>
<td>Making</td>
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<tr>
<td>Handling</td>
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<td>Replicates</td>
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<td>Flow/shear</td>
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<td>Pulse</td>
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<td>Intrinsic activation</td>
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</table>
Blood compatibility and biomarker detection

Hemobile specifics

Angular velocity (ω)

Angular displacement (θ)

Haemobile position

Test compartment

Ball valve

HaemoScan
Haemobile adjusted to heart beat frequency, Doppler flow measurement on tubing

(Tubing 8mm, position 3, 15V)
Blood compatibility and biomarker detection

Validation

• In vitro conditions (Repeatability, reproducibility, accuracy)

PDMS induced
Thrombus formation:
Detachment is possible

• Clinical effects
Validation. In vitro conditions: reproducible

Platelet adhesion to PVC (duplicates)

![Graph showing platelet adhesion to PVC](image1)

Platelet adhesion to PDMS (duplicates)

![Graph showing platelet adhesion to PDMS](image2)

Platelet adhesion to PTFE (duplicates)

![Graph showing platelet adhesion to PTFE](image3)
Validation: reproducibility of complement and donor variation

Blood compatibility and biomarker detection
Validation. Clinical effects

In vitro findings correspond with clinical observations

Examples: Heparin coated stainless steel
           Carmeda coated extracorporeal circuit
Stainless steel without coating: platelet adhesion and fibrin
Coated stainless steel, almost no deposition of blood elements
In vitro results of Carmeda correspond to clinical observations.

Terumo Duraheart stainless steel housing of LVAD after 150 days implantation in a patient.
Uncoated stainless steel stent after 1 hour blood contact in vitro: Thrombus formation
Validation. In vitro results of Carmeda correspond to clinical observations: inhibition of complement activation and leukocyte activation.

Complement activation in vitro (C5b-9)

Elastase release in vitro

Complement activation in patients

Elastase release in patients
Geometry

- Catheters, stents, vascular grafts in PVC tubing
- Heart valves: special chamber
- Other shapes: attached device
Geometry

Heart valves can be mounted in a special device with a circular inner volume.
Testing of a left ventricle supporting device. Flow is generated with the Hemobile and is applied to the test chamber.
Haemocompatibility testing ISO 10993-4

Five categories:
1. Thrombosis
2. Coagulation
3. Platelets
4. Hematology
5. Complement
Blood compatibility and biomarker detection

Choice of testing

<table>
<thead>
<tr>
<th>Thrombosis</th>
<th>Inflammation</th>
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<tbody>
<tr>
<td>SEM/Platelet adhesion/P-selectin</td>
<td>SEM/ Fibrin adhesion</td>
</tr>
<tr>
<td>count/aggregation/function</td>
<td>PTT/Thrombin generation</td>
</tr>
<tr>
<td>Release products BTG, TxB2, serotonin</td>
<td>TAT, FpA</td>
</tr>
<tr>
<td></td>
<td>Convertase activity</td>
</tr>
<tr>
<td></td>
<td>C5b-9, C3a, C5a Elastase</td>
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<td>CH50/AP50</td>
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Investigate material changes in a marketed device

- Scanning electron microscopy
- Platelet adhesion, Fibrin adhesion
- Thromboxane B2, Thrombin-antithrombin III, C5b-9, Elastase, Hemolysis

HaemoScan
Preferred direct surface examination

**Thrombosis:** Scanning electron microscopy

**Coagulation:** Fibrin adhesion

**Platelets:** Platelet adhesion, P-selectin expression

**Inflammation:**

**Hematology:** Leukocyte binding (CD11)

**Complement:** C5-Convertase or C3b

Separate experiments (24 hrs): Hemolysis
To be determined

Circulation time 4 hours, or 1 hour, or shorter time (platelet and complement react optimally within 30 minutes.)
Conclusion

In vitro systems are excellent tools to determine the material properties.

The small loop systems allow multiple testing including references with blood from 1 (human) donor.

Human blood is different from animal blood and all assays can be done on human blood samples.

Differences between donors can be observed, which may lead to an estimate of number of donors needed.

Thrombogenicity testing by in vitro systems creates new possibilities.