Hemocompatibility testing in the 21st century: Options and pitfalls

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Our research focus

**Blood contacting biomaterials / Biologization of medical devices**

- **GLP lab for hemocompatibility tests**
  1. Accredited GLP test lab for „Testing of blood contacting medical devices accord. to ISO 10993-4“ with fresh human whole blood
  2. Investigations in big animal models (pig, sheep)

- **In vitro pyrogen tests**
  Die innovative Pharmeuropa akzeptierte in-vitro Pyrogentestmethode.
  Ein humanspezifisches Verfahren zur Prüfung auf Pyrogenfreiheit von Injektabilia und Medizinprodukten

- **In vivo Endothelialization**
  Fishing for Stem cells:
  Mimicry of homing factors for in vivo cell seeding.
  Hemocompatible polymer matrix with immobilized capture molecules for EPCs.

- **Gene Silencing Medical Devices**
  Next stent generation:
  siRNA based coatings for local gene silencing to reduce neointimal hyperplasia
What is Hemocompatibility?
Hemocompatibility or subway plan of London?
Buddy’s view of the hemocompatibility problem

The catastrophe revisited: Blood compatibility in the 21st Century

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Abstract

The biomaterials community has been unable to accurately assign the term “blood compatible” to a biomaterial in spite of 50 years of intensive research on the subject. There is no clear consensus as to which materials are “blood compatible.” There are no standardized methods to assess blood compatibility. Since we use millions of devices in contact with blood each year, it is imperative we give serious thought to this intellectual catastrophe. In this perspective, I consider five hypotheses as to why progress has been slow in evolving a clear understanding of blood compatibility: Hypothesis 1—It is impossible to make a blood compatible material. Hypothesis 2—We do not understand the biology behind blood compatibility. Hypothesis 3—We do not understand how to test for or evaluate blood compatibility Hypothesis 4—Certain materials of natural origin seem to show better blood compatibility but we do not know how to exploit this concept. Hypothesis 5—We now have better blood compatible materials but the regulatory and economic climate prevent adoption in clinical practice.

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Hypothesis 1: It is impossible to make a blood compatible material.

Hypothesis 2: We do not understand the biology behind blood compatibility.

Hypothesis 3: We do not understand how to test for or evaluate blood compatibility.

Hypothesis 4: Certain materials of natural origin seem to show better blood compatibility but we do not know how to exploit this concept.

Hypothesis 5: We now have better blood compatible materials but the regulatory and economic climate prevent adoption in clinical practice.
Cardiovascular medical devices in blood contact from minutes to whole life

Art. Filter
Oxygenator
VAD
Heart valve
Stents
Vascular Grafts
What do we expect from a blood compatible surface?

Technical functionality

- No platelet adhesion
- Not thrombogenic
- Not pro inflammatory
- Pro healing
Problem

still poor hemocompatibility of the devices
"Blood is a very special juice"

Mephisto to Dr. Faust in the Pact Scene (Faust I, J.W. v. Goethe)
Blood: the well organized chaos
Hemostatic Balance

Coagulation

- Thrombosis:
  - Myocardial Infarction
  - Stroke
  - Lung Embolism
  - Deep Venous Thrombosis

Fibrinolysis

- Bleeding:
  - Cerebral Hemorrhage
  - Inner Bleeding
  - Retinal Bleeding
  - Hematoma
Hemostatic Balance

**Activators:**
- Thromboplastin (tissue factor)
- Neg. Surfaces

**Activators:**
- Kallikrein
- t-PA
- Streptokinase/r-t-PA

**Inhibitors:**
- Heparin
- Hirudin
- Hirudin
- Warfarin
- ASS

**Inhibitors:**
- Aprotinin
- PAI
- Antiplasmin

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**Coagulation**

**Fibrinolysis**
Problems Caused by Blood Contact with Artificial Surfaces

**Coagulation**
Stenosis, Thromboembolic Complications
Anticoagulants e.g. Heparin, Hirudin

**Fibrinolysis**
HLM, VADs
Proteinase Inhibitors e.g. Aprotinin

**Inflammation**
Post-Pump Syndrome
Host-versus-graft
Leukocyte Infiltration
SIRS, SEPSIS, MOF
Models for in vitro hemocompatibility testing

Static models
Dynamic models
Going from small to big models
(Wim - Hans)

Photo: J. Kozok
Hemocompatibility Testing
What kind of blood should be used?

Human whole blood
directly from healthy blood donors

1. Fresh!
2. Fresh!
3. Fresh!

Exclusion Criteria:
Smoking
Drug taking (Aspirin, Antiphlogistics, etc.)
Pregnancy, oral contraceptives
How to draw the blood?

Sterile!

No stasis, or only very short and soft stasis

Soft and slow filling

Do not produce a vacuum

Directly in containers prefilled with diluted anticoagulant

Shake them softly during donation
Need more blood for big models?

Use the transfusion service at the next corner

If not close enough:
Forget it!
What kind of blood bags can be used?

Do not use blood bags from the transfusion service!

Use empty bags prefilled with your own anticoagulant.
What kind of anticoagulation should be used?

- Use unfractionated heparin
- As less as possible or similar to clinical application
- No citrated blood
- No hirudin
- No other anticoagulants
Comparison of different heparin concentrations

Blood cell analysis

Human whole blood (n=5) was circulated in a Chandler loop model for 30, 60, 120 or 240 minutes at 37°C
Comparison of different heparin concentrations

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Thrombin generation

TAT-III complex concentration [μg/l]

Prothrombin fragment 1+2 [pmol/l]
Comparison heparin (1.0 IU/ml) vs. hirudin (50 µg/ml)

Human whole blood (n=3) was circulated in a rotator model for 30 minutes at 37°C.
What kind of predicates should be used?

- Devices which are already on the market
- with comparable surface area
- Keep background activation low!
- If you are testing small surfaces (i.e. stents)
  - Use heparin coated tubing
**Activation Markers acc. to ISO 10993.4**

**Barrel stave theory**

The most poor marker limits the overall performance

Do not use a score system

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Evaluation Procedure</th>
<th>Determination</th>
<th>Test Principle</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thrombosis</td>
<td>SEM (scanning electron microscopy)</td>
<td>Platelet adhesion and aggregation, leukocyte adhesion, fibrinogen adsorption</td>
<td>Microscopy</td>
<td>Zeiss, EVOLS 10, Oberkochen, Germany</td>
</tr>
<tr>
<td>2. Coagulation</td>
<td>Marker for thrombin generation</td>
<td>Thrombin-Antithrombin-III complex (TAT)</td>
<td>ELISA</td>
<td>Siemens Healthcare Diagnostics Products, Marburg, Germany</td>
</tr>
<tr>
<td>3. Platelets</td>
<td>Number of platelets</td>
<td>Blood cell counting</td>
<td>Cell Counter Micros 60</td>
<td>ABX Hematology, Montpellier, France</td>
</tr>
<tr>
<td>4. Hematology</td>
<td>Number of white and red blood cells</td>
<td>Blood cell counting (leukocytes, erythrocytes, Hb, HK)</td>
<td>Cell Counter Micros 60</td>
<td>ABX Hematology, Montpellier, France</td>
</tr>
<tr>
<td>5. Complement system</td>
<td>Marker for activation of the C3 complement factor</td>
<td>C3a</td>
<td>ELISA</td>
<td>Quidel, San Diego, CA, USA</td>
</tr>
<tr>
<td></td>
<td>Marker for activation of the terminal complement complex</td>
<td>SC5b-9</td>
<td>ELISA</td>
<td>Quidel, San Diego, CA, USA</td>
</tr>
</tbody>
</table>
Example for in vitro oxygenator tests

**Heart-Lung-Machine Model**

1. Uncoated oxygenator
2. Biopassive Coating
3. Bioactive Coating
Final Coagulation Cascade

- Extrinsic
  - FX
  - FXa
- Intrinsic
  - Prothrombin
  - F1+2
- AT-III
- TAT
- Fibrinogen
- FPA
- Thrombin
- Fibrin
Prothrombin fragment 1+2

[nmol/l]

0' 1' 5' 10' 20' 30' 60' 120'

Bioactive  Biopassive  Uncoated
Thrombin-Antithrombin III-Complex

[μg/l]

0'  1'  5'  10'  20'  30'  60'  120'

Bioactive  Biopassive  Uncoated
Complement System
What complement factors should be looked for?

**Comparison of C3 (immunologic) with C3a (ELISA)**

**Comparison of C4 (immunologic) with SC5b-9 (ELISA)**
C5a

[µg/l]

0' 1' 5' 10' 20' 30' 60' 120'

Bioactive  Biopassive  Uncoated
Platelet Activation
Protein Adsorption
Fibrinogen Western Blotting

1. Biopassive
2. Bioactive
3. Control
CD 41 Western Blotting

1. Biopassive
2. Bioactive
3. Control
With appropriate test models and usage of fresh human whole blood you can perfectly screen the hemocompatibility of medical devices in an early preclinical stage of device development.

Tests for quality assurance of blood contacting medical devices.

Short term animal experiments seem to be less sensitive compared to in vitro testing with fresh human blood.
Thank you for your attention!