Hemocompatibility Assessment

Determination of the interaction of blood with biomaterial surfaces

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Outline

- Topics / goals of our work
- Interaction of materials and blood – overview of reactions (short repetition)
- Experimental set-up of contact between material + blood: Incubation tools
- Parameters to identify / determine interaction of material and blood

Topics of our group

Blood activation processes

Hemocompatible Interfaces

Standardized and reliable testing methods

Blood incubation – basic requirements

- First question:
  - Relevance of test system for specific materials
  - Relevance of parameters for material use
- Fresh human blood
- No blood – air contact during incubation
- Minimal contact between test surface and blood
- Minimal contact between foreign (foreign) surfaces and blood
- Construction materials have to be hemocompatible
- Shear stress / blood flow / prevention of cell sedimentation
- Versatility for different sample geometries
- Sterilizability of construction materials
- Relevant control materials (external reference)
- Incubation at 37°C
- Duration of incubation: in accordance to designated use - blood treatment

Blood incubation – blood collection

Fresh venous human blood
Blood from blood bags (blood bank)
Animal blood
Blood treatment: anticoagulation / stabilization

Blood activation processes

- Surface functionalities impact - protein adsorption
- Adsorbed proteins impact - activation of coagulation - cell adhesion / activation
- Initiation of blood coagulation

Interactions in blood activation

Use of platelet rich plasma and washed platelets enables research on platelet interactions
Use of plasma and purified proteins enables research on coagulation enzymatic reactions
Use of isolated cells enables research on cell – surface interactions

Only whole blood with minimized anticoagulation enables us to understand the complex interactions happening after blood – material contact

Blood activation processes

- Thrombogenicity
- Inflammatory response
- Hemocompatibility

Clinical effects

- Coagulation
- Enzymatic
- Lymphocytes
- Platelet aggregation and adhesion
- Tissue destruction
- Hypersensitivity
- Specfic defense

MATERIAL SURFACE
Blood incubation models - static

Static blood incubation

- First experiments by W. Hewson 1864
- Relatively simple
- High amount of samples possible
- Almost no other materials / influences
- Negative: no physiologic blood flow

Hewson’s results:
- Blood stays liquid for a longer time if placed inside an isolated vein than if placed inside a bowl surrounding of blood influences reactions inside of blood volume

Examples:
- Incubation in open systems
- Incubation in tubes
- Incubation systems with parallel plates

Blood incubation models - static

Static blood incubation – open systems

- Very simple
- Sedimentation of cells
- Strong air contact
- Surface / blood contact not well defined

Chandler loop (developed 1958 by A.B. Chandler)

Water bath 37°C

Blood incubation models - static

- Tube is coated or small materials like stents are inserted
- Blood fill in with air bubble to allow blood “mixture” – air contact!
- No foreign materials, good relation of material to blood volume
- Only possible for coatable materials
- New development: no air but movement realized using pump

Blood incubation models - static

Special incubation chamber with defined characteristics

- Ease of handling, easy sample possible
- No air contact, good relation of sample surface and blood volume
- Spacing material: PTFE
- Blood filled in directly from syringe
- Approx. 21 parallel samples

Optimisation of chamber geometry concerning sample surface size

Blood volume similar in all chambers

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Hemocompatibility Assessment

Plate – plate incubation system

- 2 plates with test material in small distance (< 1 mm)
- Rotation of top plate

Physiologic conditions of blood flow

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Rheologic considerations for blood incubation

Shear force influences cell-surface interactions

Cell cycle should be avoided

Laminar and well defined flow conditions desirable

Model not possible to picture all physiologic conditions

Plate – plate incubation system

- Streaming channel with well defined geometry enables laminar flow and adjustable shear force (variation of channel height)
- Tapes made from hemocompatible silicon
- Only 8 parallel chambers possible

Perfusion chamber

- Building / foreign materials relatively big surface
- Slight hemolysis (destruction of red blood cells) even with lowest pumping rate
- Building / foreign materials relatively big surface

Blood incubation models

- Perifusion chamber
- Plate – plate incubation system
- Streaming channel with well defined geometry enables laminar flow and adjustable shear force (variation of channel height)
- Tapes made from hemocompatible silicon
- Only 8 parallel chambers possible
- Slight hemolysis (destruction of red blood cells) even with lowest pumping rate
- Building / foreign materials relatively big surface
Bead columns
- High material surface possible
- Possible incubation for coatable substances
- Flow conditions not well defined, turbulent and laminar conditions possible, no shear force can be defined
- Pumping and tubing needs to be considered - big surface with other materials

For dialysis materials: mini-modules
- Good correlation with end-use-product
- High contacting surface
- Pumping and tubing necessary

Analysis of blood and surface
After the incubation: blood is taken from the incubation set-up
Blood and surface will be analyzed

Parameters for hemocompatibility
What do we want to know?

Analysis of blood chambers
1. Anticoagulation of blood
2. Analysis of blood before incubation
3. Incubation at 37 °C
4. Analysis of blood and materials surface

Blood analysis - ELISA
Parameter determined using ELISA assays (our experimental set-up)
- Coagulation activation: TAT
- Complement activation: C3a, C4a, C5a, C6
- Platelet activation: PAI, g-TX
- Leukocyte activation: Elastase, TNF

All of these are proteins or peptides in small quantity to be found in a very complex milieu

Blood analysis - ELISA
ELISA: enzyme linked immune sorbent assay
- Antibodies immobilized to surface of microtiterwell
- Plates a contact microplate, antibody is bound to antibody
-enzymatic reaction can bound substances
- Second antibody is enzyme linked
- Amount of dye product reflects surface concentration of bound analyte

Determination of blood cell numbers using Coulter Counter ActDiff.
Measuring principle: Counts and measures cells
Detects change in electrical conductance (impedance)
Photometric measurement for hemoglobin after cell lysis

Blood analysis - ELISA
Blood cells measured in two chambers filled with conductive saline fluid, separated by a small orifice

2 electrodes

Blood analysis – Cell counting
Parameters:
- Leukocyte numbers (lymphocytes, monocytes, granulocytes)
- Platelet numbers
- Average cell sizes
- Red blood cells
- Hemoglobin
- And more.......

Blood analysis - ELISA
Coagulation activation: TAT
Complement activation: C3a
Platelet activation: PAI
Blood analysis – FACS
Flow cytometry also called FACS: fluorescence assisted cell sorting
- Hydrodynamic focusing
- Size and granularity of cells lead to differences concerning light refraction
- Fluorescence used to differentiate cell characteristics

Blood analysis – enzyme activity
Enzyme activity after blood contact can be tested:
- Using specific chromogenic or fluorogenic substrates
- Using natural substrates like e.g. fibrinogen for thrombin activity

Surface analysis – protein adsorption
Determination of adsorbed proteins using specific antibodies
Dry / frozen surface contacts buffer with specific antibodies
Example: complement fragment C3c or fibrinogen/fibrin
Use of (second) conjugated antibody
Enzymatic (peroxidase)

Surface analysis – SEM
SEM: Scanning electron microscopy
Sample surface rinsing
Surface fixation using glutaraldehyde (stabilization of structures)
Dehydration using alcohol
Optional: critical point drying (samples are analyzed in vacuum)
Sputter coating with gold (samples need to be conductive)

Surface analysis – Fluorescence microscopy
Determination of surface cell density AND state of activity possible

Fluorescence intensity: one cell = one count

thrombin + fibrinogen
Optical measurement of fibrin formation

Stages of platelet adhesion / activation

Platelets
PTFE CD41a
Platelet
PTFE CD62p
Self assembled monolayers (SAM) as model surfaces to study initial processes of blood coagulation

- aliphatic thiol on gold with various functional end groups
- excellent model to study surface chemistry related blood reactions

From research to product

In vitro results from experiments → promising products
Enhanced in vitro experiments in accordance with legal requirements regulated in the EU / Germany through harmonised standards and laws (Medizinproduktegesetz)
(ISO 10993, coordination of chosen tests with notified body
Possibly animal experiment
In vivo studies
Release of certified new product

Take home message

Interaction between material and blood is governed by cellular and humoral reactions that may lead to coagulation and inflammation.

Development of enhanced materials with better hemocompatibility needs sensitive testing with relevant blood incubation and adapted selection of parameters.

Several basic requirements should be respected for planning a blood incubation set-up. The final application should be kept in mind.