Surface Endotoxin Contamination:
Quantification and Influence on Hemocompatibility Evaluation

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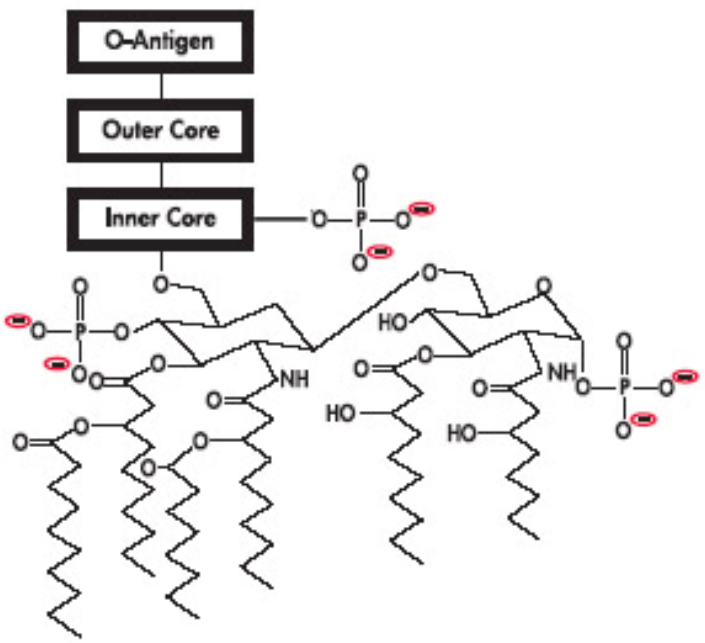
Endotoxin - Background

- Compound of gram negative bacteria cell wall
- Structurally Lipopolysaccharides
  - Hydrophobic and charged hydrophilic compounds
- Adsorption to hydrophobic and positively charged surface
- Ubiquitary present as contamination in liquids and on surfaces



(adapted from Qiagen)

Biological Response to Endotoxins

- Stimulation of inflammatory cells
- Cytokine release
  - IL-1, IL-6, TNF
- *In vivo*: fever

- Complement activation
- Coagulation activation

Objective

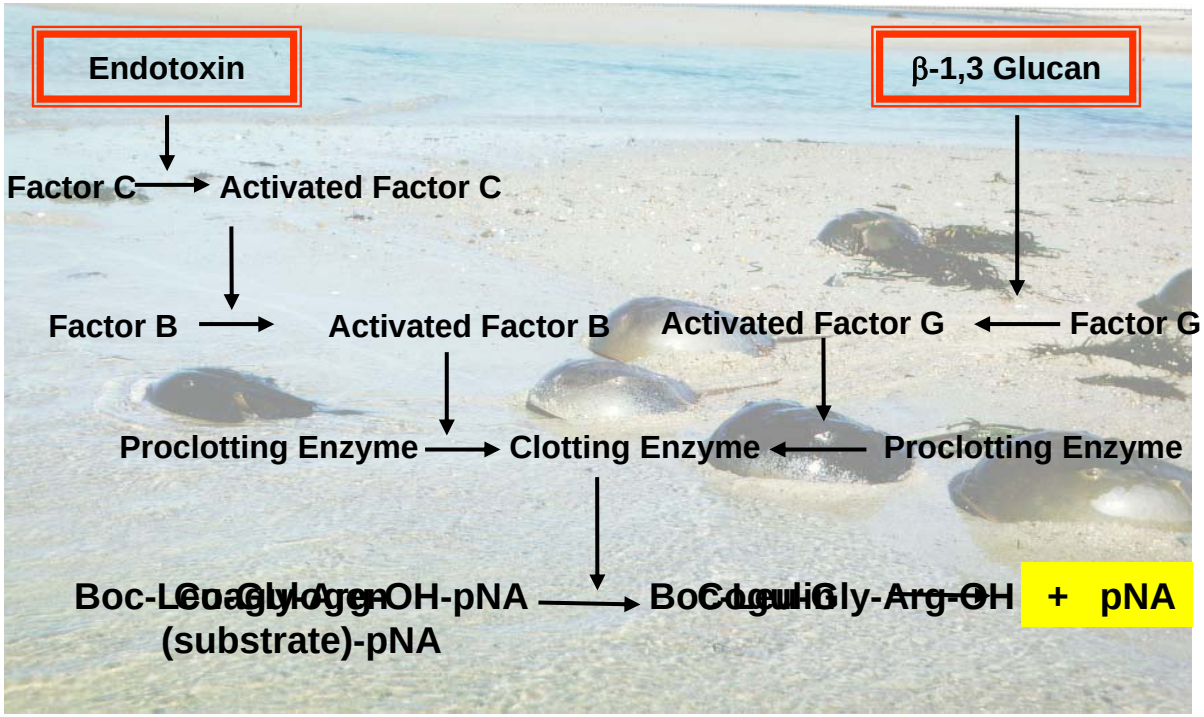
Biomaterials research requires discrimination

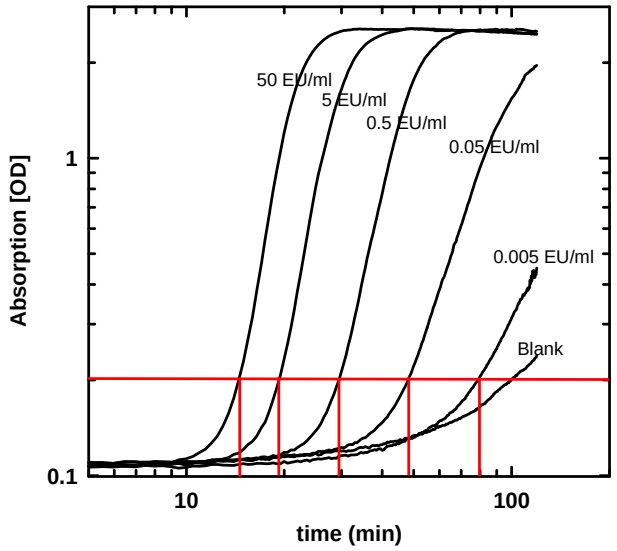
- Materials-specific inflammatory response
- Endotoxin-induced inflammatory response

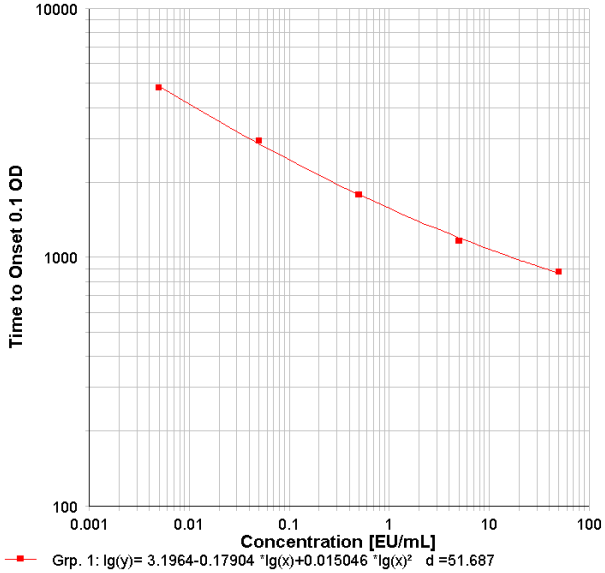
Or

- Verification/exclusion of surface endotoxin contamination

Limulus Amebocyte Lysate (LAL) Assay







A chromogenic, **kinetic** LAL test is the standard assay for specific endotoxin quantification

FDA Regulations

Endotoxin Contamination of Medical Devices

- Water-Extracts are tested. It is assumed that <50% of adsorbed LPS go into the liquid phase
- Extract conditions
  - Typically extract with 40ml water
  - 1 hour at room temperature (>18°C) or 15min at 37°C
  - “For very big or small devices the volumes has to be adjusted”
- Concentration in Extracts
  - < 0.5 EU/mL for peripheral devices
  - < 0.06 EU/mL for devices with contact to cerebrospinal liquor
  - (1 ng E.coli O55:B5 LPS = 5 EU)

Problems:

- Low solubility in water does not allow complete elution
- Dependence of elution on surface properties is unclear.

Procedure

Use of the approved liquid phase LAL assay for determination of surface adsorbed LPS

- LPS elution from the surface and separate testing
  - Influence of elution media?
  - Quantitativity of the assay?
  - Sensitivity to other surface properties?
- Execution of the assay in direct contact
  - Quantitativity of the assay?
  - Sensitivity to other surface properties?
- Hemocompatibility assay of endotoxin contaminated surfaces

LAL Sensitivity to Solvents and Detergents

Objective

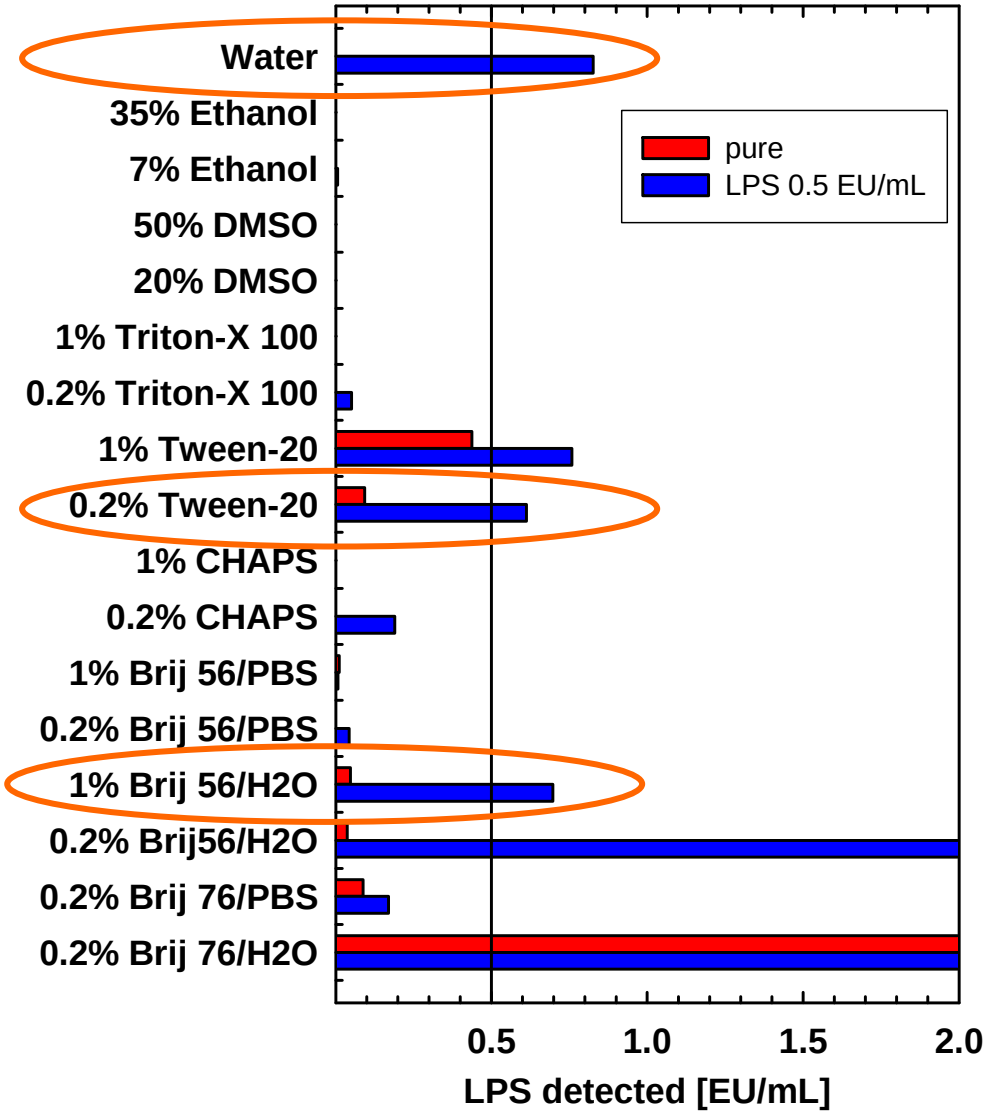
- Interaction of organic solvents and detergents with the LAL system

Method

- Determination of baseline LAL activation and activation with defined LPS concentrations

Result

- Strong activating or inhibiting interference by most media.
- 0.2% tween-20 or 1% Brij 56 in water allow quantitative detection of the spiked endotoxin



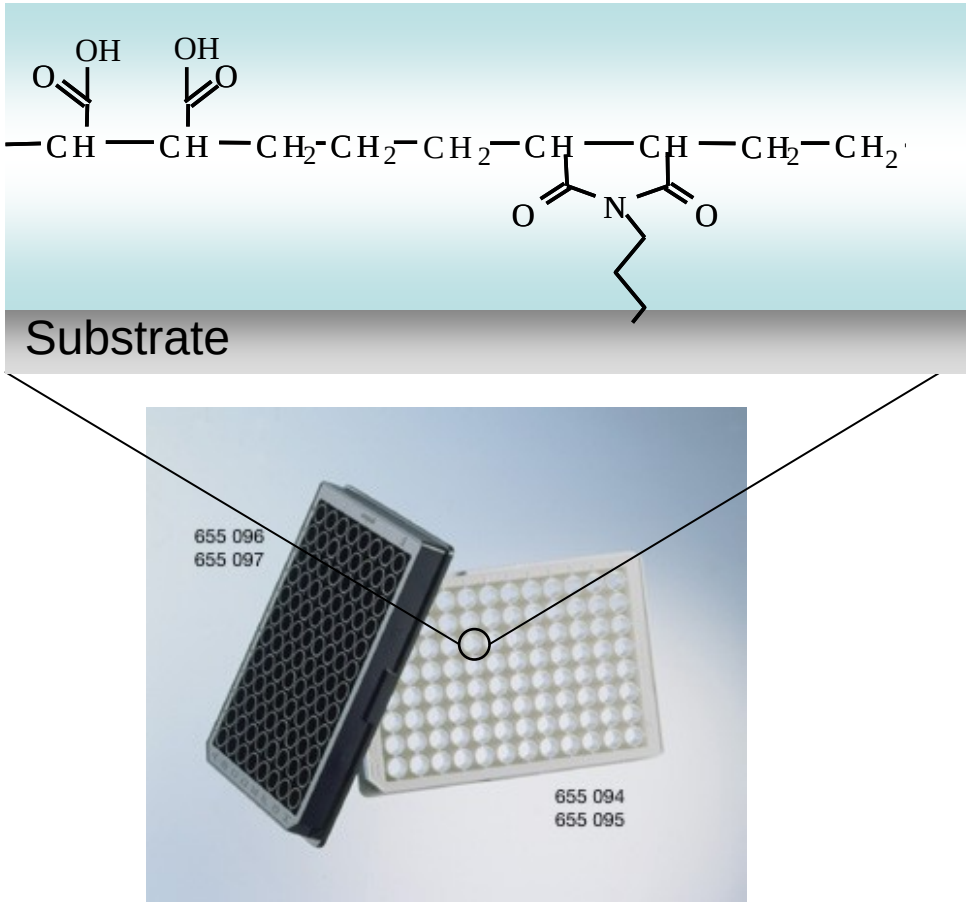
Evaluation of Surface Adsorbed LPS

Objectives

- Elution of LPS with different solvents or run of LAL test in contact with contaminated surface

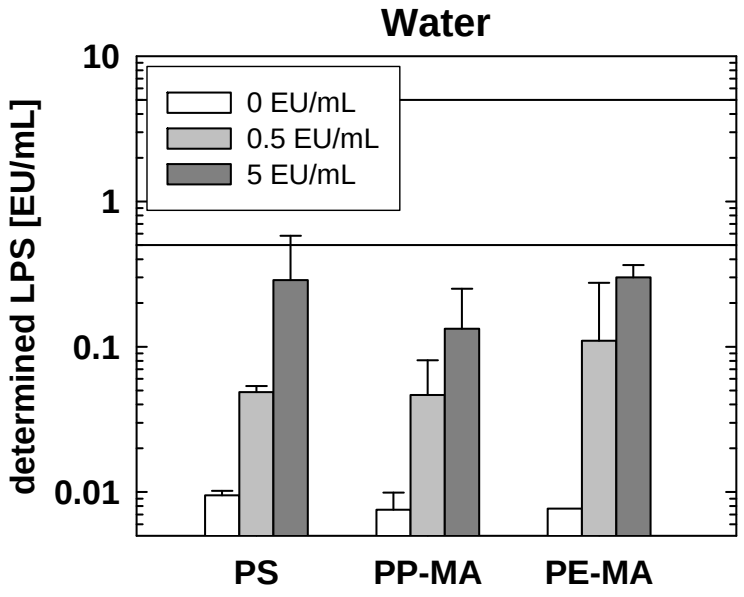
Method

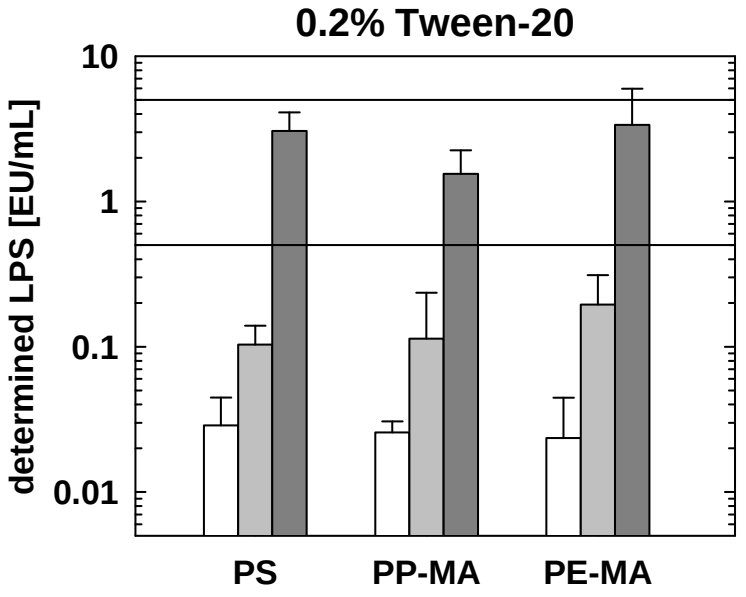
- Multiwell-plate (polystyrene) was coated with PE-MA or PP-MA co-polymers
- Spiking of the wells with defined LPS concentrations from alcohol solution
- Elution of the adsorbed LPS (60min, RT)
  - Water
  - 0.2% tween-20
  - 1% Brij 56
- LAL assay with these eluates in clean wells
- LAL assay directly in spiked wells.

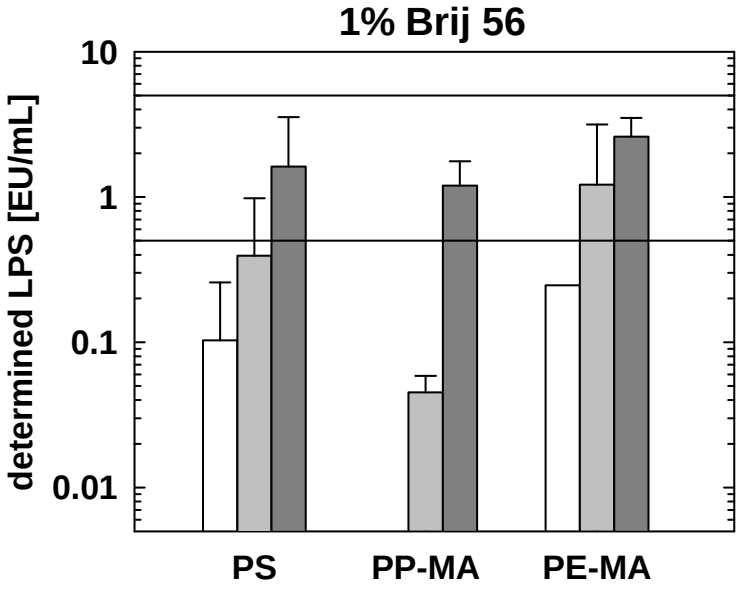


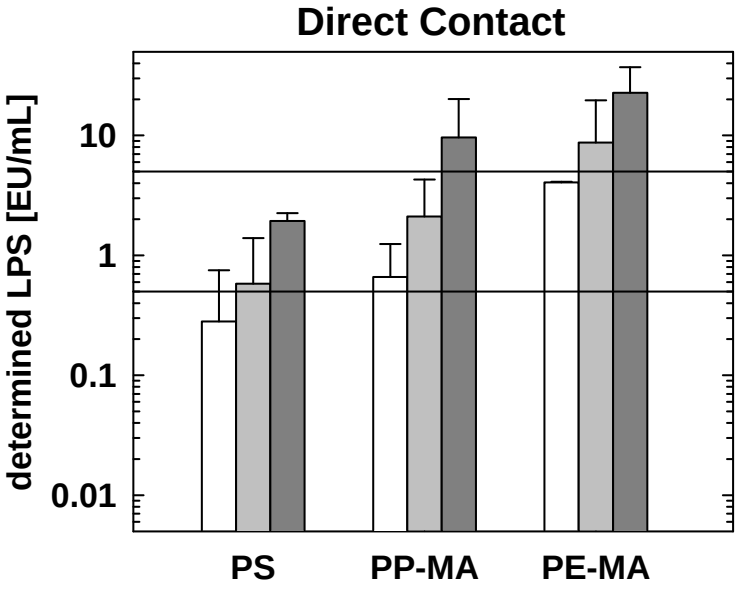
Material	Contact Angle
PS	76°
PP-MA	38°
PE-MA	21°

Detection of Surface Adsorbed LPS





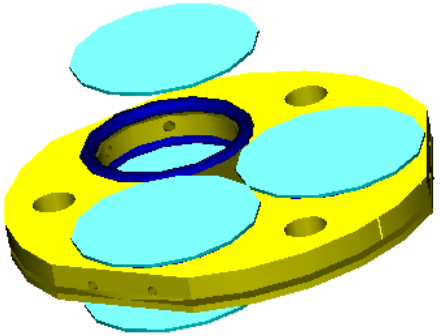




Influence on Hemocompatibility Evaluation

Ojective

- Evaluation of the biological activity of surface adsorbed LPS in short term assay



Incubation Chamber

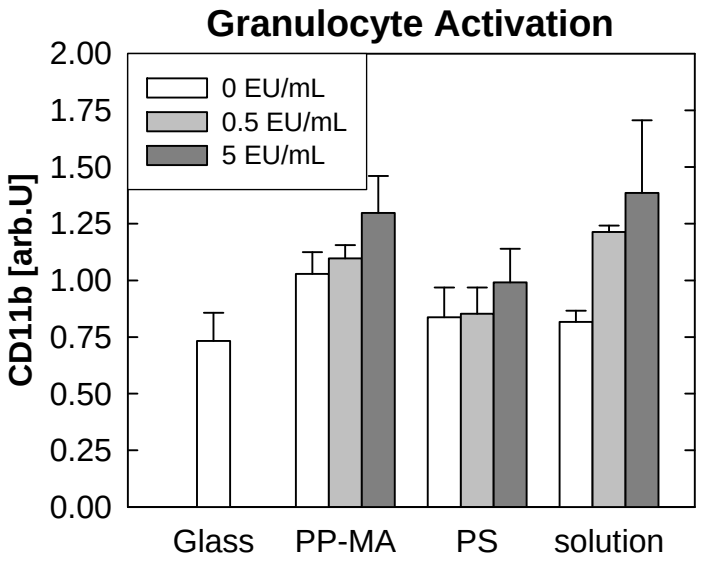
Method

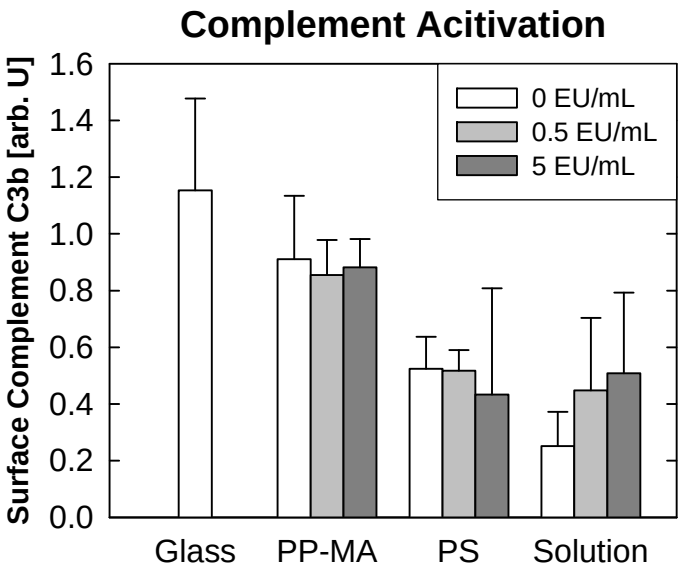
Samples	LPS
PP-MA PS (liquid)	0 EU/mL 0.5 EU/mL 5 EU/mL

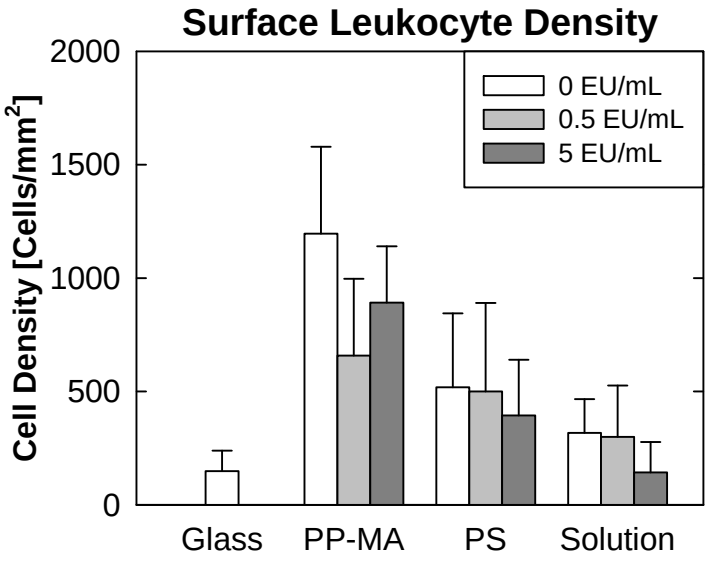
- Incubation with whole blood
- Incubation time 2 hours
- Analysis of inflammation and hemostasis markers by standard techniques

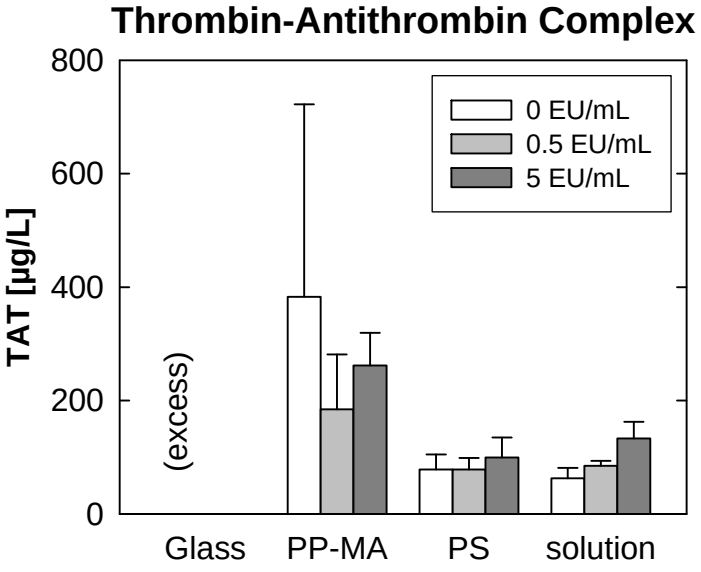
Inflammation	Hemostasis
Surface C3b	TAT complex
Complement C5a	Surface fibrinogen
Leukocyte CD11b expression	Platelet factor 4 release
Leukocyte surface density	Blood platelet decay

Influence on Hemocompatibility







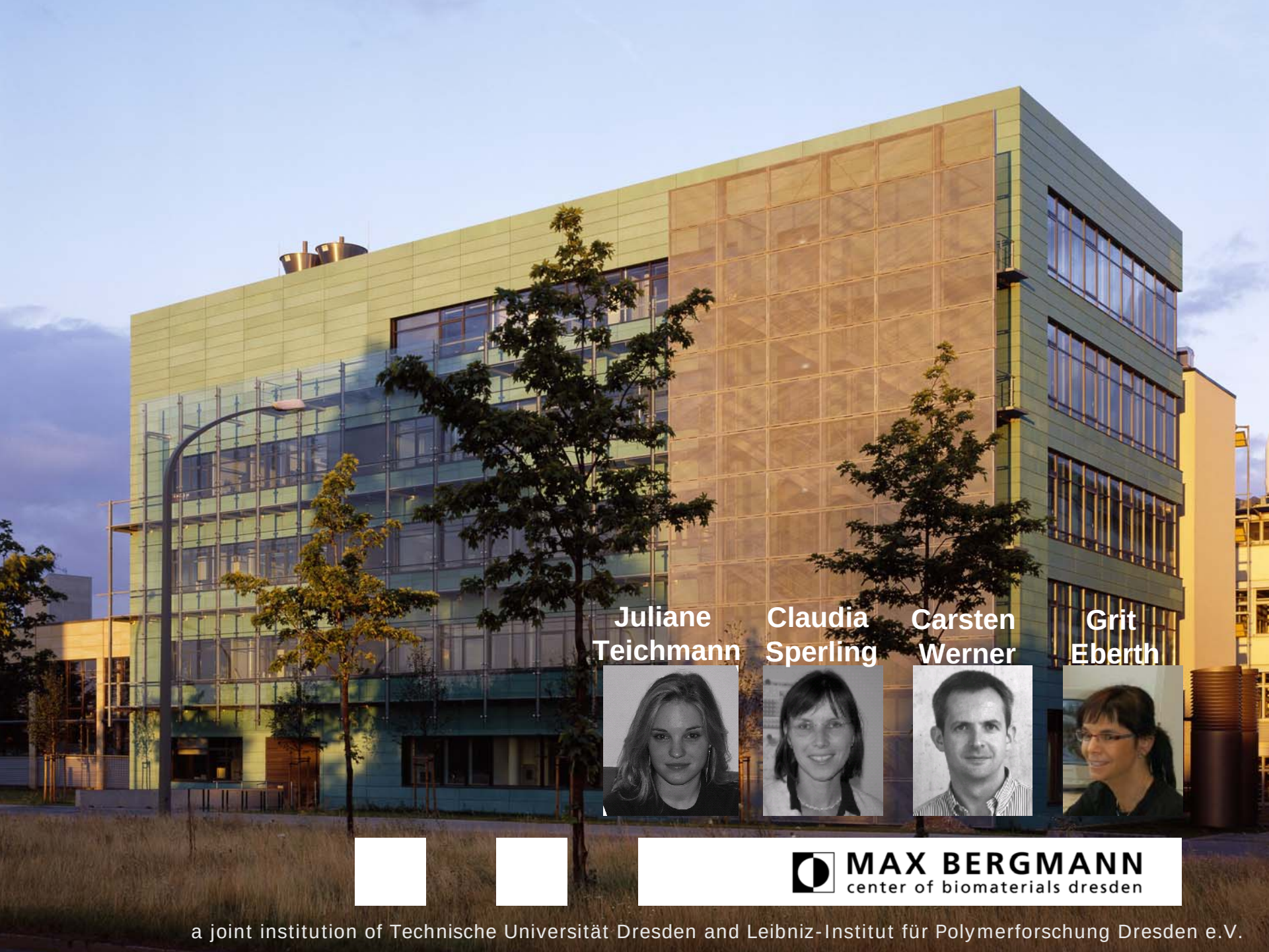


Summary and Conclusion

- Wide prevalence of endotoxin contamination
- Similarity of hemo-*in*compatibility and endotoxin-related effects
  - Separate evaluation of the effects is mandatory
- Liquid phase LAL assay is sensitive to many organic solvents and detergents
  - 0.2% Tween-20 and 1% Brij 56 have only little interference
- Elution with 0.2% Tween-20 allows almost quantitative detection of surface adsorbed LPS in LAL assay and is mainly independent of surface properties
- Hemocompatibility assays with short (two hours) exposure time are more sensitive to materials properties than to endotoxin contamination

Open Questions and Outlook

- How big is the time window for the blood compatibility test which allows this discrimination?
  - Definitely below 16-24 hours but not closer determined.
- How much information of biocompatibility is lost when performing the assay within this timeframe?



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