

Analysis of Surface Endotoxin Contamination and Influence on Hemocompatibility

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Background

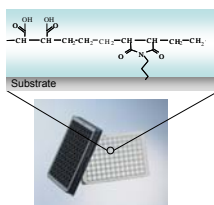
- Endotoxins (LPS) are frequent contaminations on surfaces and difficult to remove due to their hydrophobic character.
 - Limulus ameocyte lysate (LAL) assay is an approved, specific and sensitive test for endotoxin quantification in liquids, but not approved for surfaces.
 - Biological effects of endotoxins are non-specific inflammation and mainly *not* distinguishable from bio-*in*compatibility effects.
- ⇒ Biomaterials research requires
- Verification/exclusion of surface endotoxin contamination
 - Discrimination: Materials-specific ↔ Endotoxin-induced inflammatory response

Methods

Quantification of Surface Endotoxin

Endotoxin spiking of surfaces with different surface properties. LAL assay of eluates and in direct contact.

- Plate (polystyrene) coated with maleic acid anhydride (MA) co-polymers
- Spiking with defined LPS concentrations
- Elution of the adsorbed LPS with preselected media (60 min, RT)
 - Water
 - 0.2% Tween-20
 - 1% Brij 56
- LAL assay of these eluates in clean wells
- LAL assay directly in spiked wells

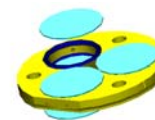


Material	Wetting Angle
PS	76°
PP-MA	38°
PE-MA	21°

Influence of Surface Endotoxin on Hemocompatibility Evaluation

Incubation of whole blood with endotoxin adsorbed on different surfaces or with free endotoxin.

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

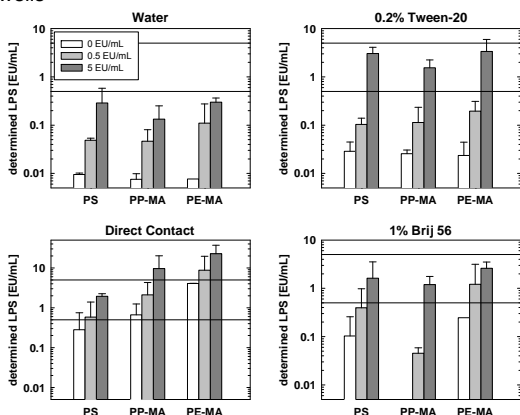


Incubation Chamber

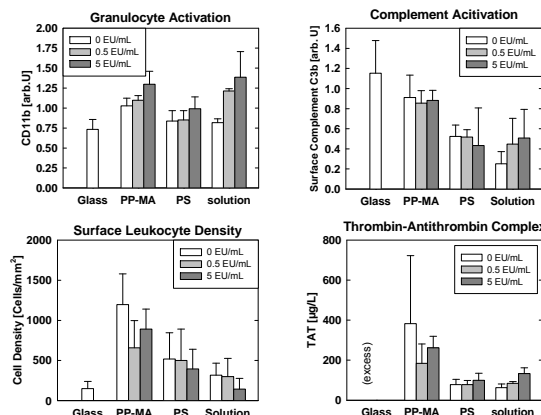
- Incubation time 2 hours
- Analysis of inflammation and hemostasis markers by standard techniques

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

Results



- Water: Low elution and apparent saturation effects (lower elution rate for higher concentrations)
- Direct contact: High dependence of the of the detection rate on surface properties
- Detergents: Tween-20 produces better reproducible and better graded results than 1% Brij 56



- Endotoxin in solution: Obvious sensitivity of coagulation and inflammation
- Surface adsorbed endotoxin: Little dependence of coagulation and inflammation
- Different material properties: Higher sensitivity of the assays to materials properties than to adsorbed endotoxin

Summary/Conclusion

- Similarity of hemo-*in*compatibility and endotoxin-related effects requires separate evaluation of the effects.
- 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.

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Introduction: Endotoxin (LPS) contamination on surfaces has high prevalence. Biologically, endotoxins induce non-specific inflammatory reactions and due to their structure they are very resistant to cleaning methods. On surfaces for biocompatibility evaluation, such as hemocompatibility testing, endotoxin contamination may cause misleading results, because materials-specific reactions and reactions due to contamination look similar.

Separate analysis of endotoxin contamination and hemocompatibility therefore is mandatory, and endotoxin determination has to be unbiased by surface properties. The limulus amoebocyte lysate (LAL) assay is the standard assay for LPS detection in solution phase, however the application for surface-adsorbed endotoxin is challenging and not yet validated. The adsorbed endotoxins either have to be eluted from the surface for analysis, or the assay needs validation at the solid phase.

Materials and Methods: A three-step approach was chosen:

- Analysis of the interference of different solvents (water, ethanol, DMSO) and detergents (Triton-X 100, Tween-20, Brij series, CHAPS, dissolved in PBS or water) with the LAL assay.
- Elution and detection of defined endotoxin amounts from surfaces with various properties using selected elution media or execution of the assay in direct contact with the spiked surface.

Polyethylene and polypropylene maleic acid copolymers (PE-MA and PP-MA, respectively) were coated on polystyrene (PS) microplates, leading to water contact angles of 21° (PE-MA), 38° (PP-MA), and 76° (PS). Endotoxin was adsorbed from an ethanol solution on the surfaces and eluted with ultrapurified water, 0.2% tween-20 or 1% Brij-56 and the LAL assay was performed subsequently with the eluates or the assay was performed directly in the spiked wells. Expected LPS concentrations were 0, 0.5, and 5 EU/mL.

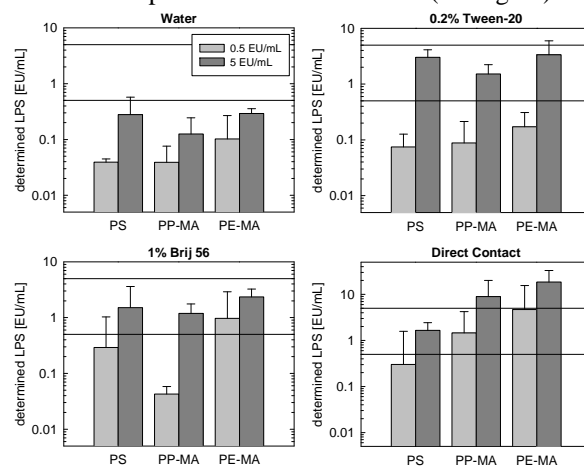
- Hemocompatibility evaluation of surfaces with different baseline hemocompatibility spiked with LPS.

Two hours incubation of whole blood with PS or PP-MA surfaces spiked with LPS to achieve the concentrations 0, 0.5, and 5 EU/mL and separately blood spiked with the same LPS concentrations in contact with relatively inert surfaces (PTFE). Evaluation of inflammation parameters (leukocyte CD11b expression, surface complement C3b, plasma C5a) and coagulation parameters (TAT complex, surface fibrin(ogen) deposition).

Results and Discussion: Organic solvents at concentrations typically applied for extraction strongly interfered with the LAL assay: Spiked endotoxin was not detected.

Non ionic detergents and the zwitterionic detergent CHAPS partly inhibited and partly enhanced the chromogenic LAL reaction without clear structure function relation. 0.2% tween-20 or 1% Brij-56 in water had least influence on LPS determination and were applied in the further steps.

The LAL test performed in direct contact with LPS contaminated surfaces overestimated the LPS amount on hydrophilic surfaces and underestimated the amount on hydrophobic surfaces. Further, it did not react linearly on different concentrations. LPS release with water detected less than 10% of the expected concentration with little dependence on surface properties, however, lower concentrations were eluted at higher efficiency than higher concentrations. The elution with Brij-56 was highly dependent of surface properties and concentration, whereas elution with 0.2% Tween-20 best presented the expected LPS concentration (see figure)



Detected LPS concentration after elution of defined amounts of surface adsorbed endotoxin. Horizontal lines indicate the expected concentrations of 0.5 and 5 EU/mL.

In blood incubated with LPS adsorbed surfaces the endotoxin had only minor effect on inflammatory or coagulation parameters, however inflammation was highly sensitive to soluble endotoxin. The tests also were sensitive to surface properties of the materials.

Conclusions: Surface adsorbed endotoxin can be eluted for analysis in a chromogenic LAL assay with 0.2% tween-20. This elution is relatively independent of the hydrophobicity of the material. The efficiency of elution with water or analysis in direct contact is more dependent of the adsorbed concentration and of surface properties.

Hemocompatibility assays with incubation time not exceeding two hours are more sensitive to materials related properties than to the adsorbed LPS amount. Analysis of hemocompatibility therefore can be performed unaffected by endotoxin contamination.