Neutrophil extracellular traps on biomaterials?

Claudia Sperling, Marion Fischer, Carsten Werner.
Max Bergmann Center for Biomaterials, Leibniz-Institute of Polymer Research Dresden

Statement of Purpose: Immune reactions upon material-blood contact are initiated through complement activation leading to activation and adhesion of neutrophil granulocytes. Newly discovered neutrophil extracellular trap (NET) formation by neutrophil granulocytes represents a defense mechanism that includes neutrophil chromatin release binding invasive bacteria. We speculate that NET formation is also initiated on biomaterials surface as we found disintegrated DNA on hydrophobic surfaces after *in vitro* whole blood incubation.

Neutrophil granulocytes reactivity is an important aspect for the hemocompatibility of biomaterials because activated neutrophils can also trigger coagulation activation on biomaterials. In the past we studied the relevance of tissue factor for this interaction.[1] The interaction of recently discovered neutrophil extracellular trap formation (NETs) with coagulation and platelet activation provoked the hypothesis of a relevance of this reaction for biomaterials' interaction with blood. NET formation independent of pathogenic triggers was already reported. Bartnek et al. found NET participation for the clearance of nanomaterials with differing intensity depending on surface composition.[2]

Methods: Hemocompatibility of materials (here PTFE (ElringKlinger Kunststofftechnik GmbH, Germany)) was determined in *in vitro* testing using heparinized (2 IU/ml) whole human blood and in house designed incubation chambers. NET formation of isolated granulocytes (density gradient centrifugation using Polymorphprep / Lymphoprep (Axis-Shield, Norway) followed by Percoll [3] (GE Healthcare, Germany)) was studied on glass and on model surfaces with controlled properties (SAM surfaces with hydrophilic –OH and hydrophobic –CH_3 endgroups). Citrated pooled blood plasma was incubated prior to surface incubation with isolated cells (30 min @ 37°C) if indicated. Granulocytes were incubated for 1 hour @ 37°C w 5% CO_2 before activation using PMA (600 nM) and evaluated by fluorescence microscopy studying chromatin decondensation (staining of DNA with DAPI), citrullinated histones (Anti-Histone H3 ab 80256, abcam, Great Britain) and neutrophil elastase (ab21595, abcam).

Results: Our hemocompatibility assays include the evaluation of leukocyte adhesion on biomaterials and regularly show disintegrated granulocyte nuclei on the hydrophobic reference material PTFE. These structures resemble neutrophil extracellur traps.

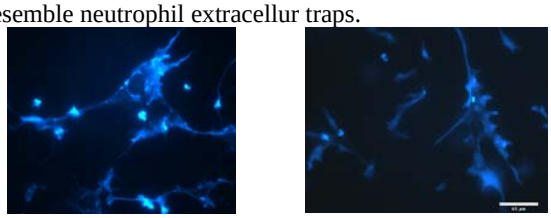
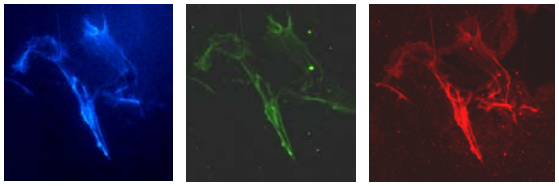


Figure 1. DNA (DAPI stained) on PTFE after *in vitro* blood incubation with fresh whole human blood

As a first step for a systematic analysis of a possible NET formation we incubated isolated granulocytes on biomaterials with and without prior adsorption of proteins from pooled human plasma. Without prior adsorption of proteins cell adhesion was negligible on hydrophilic OH SAMS but strong on glass and hydrophobic SAM surfaces. The induction of NET formation using PMA was weak after 15 min on all surfaces but effective after 3 hours for all surfaces. Without PMA cell morphology was not altered on OH SAMs and glass but on CH_3 SAM surfaces cell flattening resulted for appr. 20% of cells.

Further experiments with pre-adsorbed plasma proteins showed strong cell adhesion on hydrophilic SAMS and glass yet heterogeneous and less dense cell adhesion on CH_3 surfaces. But while NET formation was only visible on hydrophilic samples after PMA treatment several cells showed NET like structures on hydrophobic SAM surfaces after 4 hours of cell adhesion (figure 2) w/o PMA addition.



DNA histon H3 elastase

Figure 2. Surface adherent granulocytes on CH_3 self assembled monolayer showing DNA (left: blue), histon H3 (middle: green) and elastase (right: red)

Conclusions: The presence of neutrophil extracellular traps on biomaterial surfaces presents another possibility of the immune system to react towards foreign surfaces. Up to now we only studied a limited amount of materials and found NET formation on materials (in absence of other activators) solely on hydrophobic surfaces. As these surfaces generally do not support leukocyte adhesion to a greater extent, the relevance of this reaction needs to be studied with greater emphasis. The release of ROS is another parameter that could provide additional information on the intensity of NET formation.

References: Fischer M. Biomaterials 2010;31:2498-507
2 Bartneck M. Nano Lett. 2010;10:59-63
3 Brinkmann V. J Vis Exp. 2010:e1724