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Relevance of tissue factor for biomaterial associated blood coagulation

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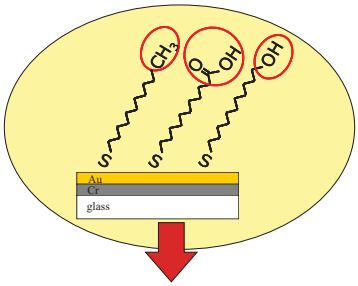
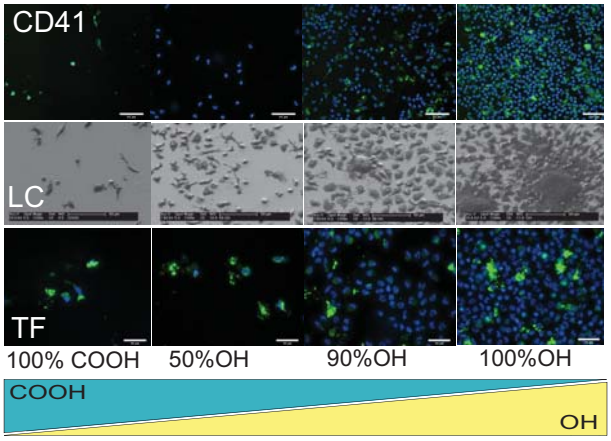
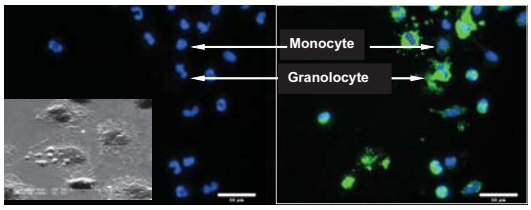


Materials and methods

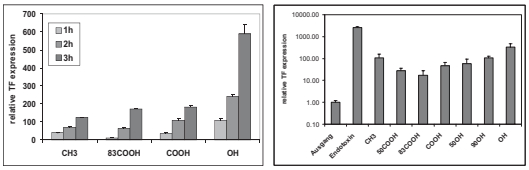
Model surfaces: Self assembled monolayers on gold surfaces were prepared from 11-Mercaptoundecanoic-acid (MUA/C_{10}-COOH) and Undecanol (C_{11}-OH). Single component SAMs named **0% -OH** (100% C_{10}-COOH) and **100% -OH** (100% C_{11}-OH) were prepared, as well as binary layers with C_{10}-COOH/C_{11}-OH-ratios of 50/50 (**50% OH**) and 10/90 (**90% OH**) .
The surfaces were incubated with fresh human blood (heparinized) for 1....3 hours. Following this incubation plasma, cells and surfaces were analysed concerning the activation of coagulation, complement, platelets and leukocytes as well as for tissue factor (TF) presentation.

Incubation of SAMs with human blood plasma or whole blood





Presence of TF (green) is associated with monocytes AND granulocytes (blue): Platelets as well as microparticles derived from monocytes and / or platelets adhere to activated leukocytes



Level of TF mRNA in leukocytes after incubation of whole blood on -COOH/-OH-terminated SAMs relative to initial value; left: expression after 1, 2 and 3 hours of blood incubation; right: expression after 2 hours of blood incubation (endotoxin induced expression was 2629 ± 234)

Motivation

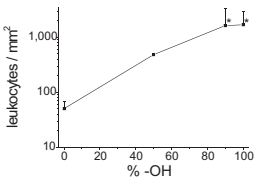
The hemocompatibility of biomaterials still presents a challenge for the usage of biomedical devices. Chemical-physical surface properties such as electrical charge and wettability determine the fate of blood proteins, enzymes and cells. Cascade reactions (coagulation, complement) and cell adhesion phenomena are strongly influenced by differences in these reactions. Self assembled monolayer (SAM) as well defined model surfaces enable the definition of surface properties on a quasi molecular level. This enables the study of initial processes at the blood-biomaterial interfaces presented here.

Biomaterial induced thrombus formation usually is attributed to the activation of the contact cascade of blood coagulation and/or the activity of adherent / activated platelets.
Yet *in vivo* tissue factor (TF) is the main initiator of blood coagulation. Since it is known now that TF also is present in circulating blood and that TF activation in blood cells can be induced by a variety of stimuli a role of this process on biomaterial surfaces appears possible.

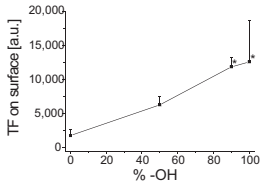
Aim

To clarify the influence of biomaterial surfaces in blood contact for the expression of TF.

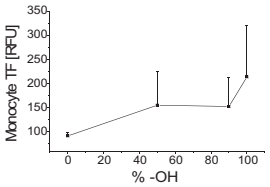
To resolve the following questions:
How do biomaterial related differences for TF expression relate to other blood activation processes ?
Are there TF related differences in blood coagulation processes?



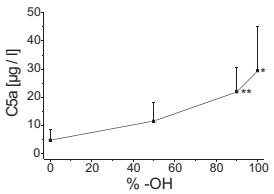
Number of adherent leukocytes after incubation of SAM surfaces with whole blood for 2 hours. Determination by fluorescence microscopy after DAPI staining of cell nuclei (*Values statisticically significant compared to 0% and to 50% -OH)



TF on adherent cells after incubation of SAM surfaces with whole blood for 2 hours. Intensity of fluorescence measured by FLA imager after FITC-anti TF staining on SAM of -COOH/-OH (*Values statisticically significant compared to 0% -OH)



FACS analysis of tissue factor presence associated with monocytes after incubation of SAM surfaces with whole blood for 2 hours.

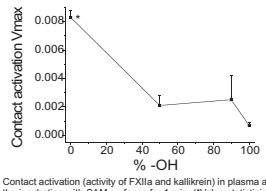


Complement activation. Detection of complement fragment C5a in plasma using ELISA after whole blood incubation for 2 hours (*Value statisticically significant compared to 0% and 50 % -OH, ** value compared statistically significantly different from 0% -OH)

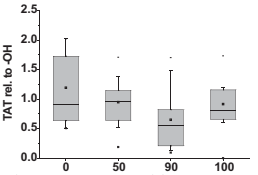
Results

The presence of TF in blood and on surface adherent cells after an *in vitro* incubation of fresh whole human heparinized blood on model surfaces was shown. The surfaces were smooth and hydrophilic but varied concerning their electrical charge. The surface with 100% -COOH (=0% -OH) was negatively charged while the surface with 100% -OH was not. There were two additional surfaces combining these characteristics (50% and 90% -OH). We could see that the presence of negative charge as well as of hydroxyl groups lead to explicitly varied reactions:

- Correlation of TF mRNA transcription with the content of -OH groups
- Increase of TF mRNA levels over time for at least three hours
- Leukocyte adherence significantly enhanced on surfaces with 90% and 100% -OH groups
- Enhanced presence of TF on surface adherent cells for surfaces with 90% and 100% -OH groups
- Monocytic TF presentation on cells freely suspended in blood: higher for surfaces with a rising content of surface -OH groups
- Complement activation (concentration of complement fragment C5a) was elevated in blood after the incubation on 90 and 100% OH-groups
- Contact activation significantly enhanced on the surface with a maximum density of negative surface charges (0%-OH)
- Activation of coagulation (formation of thrombin-antithrombin-complex TAT) not significantly different between the samples



Contact activation (activity of FXIIa and kallikrein) in plasma after the incubation with SAM surfaces for 1 min. (*Value statisticically significant different compared to 50, 90 and 100% -OH)



TAT (thrombin-antithrombin-complex) formation in plasma after whole blood incubation with SAM surfaces. Graph shows mean value, median, 25 and 75% percentile as well as 90 and 10% value)

Conclusion

TF mRNA transcription as well as the expression of TF on cells showed relevant differences in relation to the composition of the incubated material. The weakest TF presence for all tested parameters was seen for the surfaces with no -OH groups. The presence of -OH groups enhanced the activation of the complement system as well as the adhesion and activation of granulocytes and monocytes. It is known that several proinflammatory mediators induce TF expression *in vitro*. For our experimental set-up the activation of complement leading to an enhanced adhesion and activation of granulo-and monocytes seems to be responsible. The coagulation activation did not correlate with the activation of the contact cascade which was strongest on the 100% -COOH surface nor with the expression of TF. As these results only reflect the *in vitro* situation after 2 hour, the relevance of TF related thrombus formation on biomaterials *in vivo* still is likely.

PP1.3-6

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Objectives: The initiation of blood coagulation on biomaterial surfaces usually is attributed to the activation of the contact phase. Tissue factor (TF) up to now is not thought to be relevant for material associated blood coagulation. Regarding new insights into the presence of TF in whole blood this should be reconsidered.
Design and methods: Model materials with clearly defined surface groups (Self assembled monolayers of alkylthiols (SAMs) displaying various ratios of -CH3, –OH, and –COOH terminations) were used for studying the relevance of surface properties for the initiation of blood coagulation. An in vitro assay using fresh heparinized whole human blood was used to determine blood reactivity and TF expression and release.
Results: The transcription of TF mRNA showed clear differences related to surface properties and increased over time for up to 3 hours (relative expression to initial: hydrophobic -CH3: 125±1; negatively charged -COOH: 181±11; hydrophilic –OH: 590±50) A positive correlation between TF transcription and presence on leukocytes (microscopic analysis using antibody to VIC7), leukocyte activation (CD11b on granulocytes) and complement activation (C5a in plasma) was shown. A correlation between coagulation activation (plasmatic TAT) and TF mRNA was not yet found in our experimental model.
Conclusions: Material related differences for TF transcription and release were found. These differences did not relate to the activation of coagulation as studied, which might relate to methodological limitations like the short incubation time (2 to 3 hours). This set up will be further optimised and more TF related parameters will be analyzed.