Utilization of thiol-modified surfaces to investigate contact activation of plasma coagulation

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Introduction: Coagulation resulting from contact activation due to blood-material interactions remains a challenge in the use of blood-contacting cardiovascular materials. The current widespread view of contact activation imparts coagulation factor XII (FXII) activating abilities specific to anionic hydrophilic surfaces. However, previous studies from this group observed nearly equal levels of surface-mediated autoactivation of FXII at both hydrophilic and hydrophobic surfaces [1]. Further, FXIIa generation in plasma is found to be attenuated at hydrophobic surfaces rather than accentuated at hydrophilic surfaces [1,2], thus the need to further explore the role of the surface in contact activation. A mathematical model of material-induced coagulation was previously developed to study the role of the material [3]. The model utilized silane-modified glass beads as procoagulants in in vitro coagulation assays. The use of silane-modified glass beads imparts practical limitations on the types of surfaces studied. Chief among these limitations is the ability to impart spatial chemical distributions on the glass bead surfaces. Previous investigations by this group suggest that surfaces with nanoscale chemical heterogeneity affect the plasma coagulation response [4]. However, efforts to construct a more diverse group of surfaces with nanoscale chemical heterogeneity in order to investigate these responses have proven difficult. Creating a similar system utilizing thiols would allow for a more diverse group of surfaces to be created and the plasma coagulation response to spatial chemical distributions studied.

Statement of Purpose: In this study thiol-modified surfaces were prepared and utilized as the procoagulant for in vitro coagulation assays. As a first step, hydrophobic and anionic hydrophilic thiol surfaces were prepared and their performance in in vitro coagulation assays compared to their silane-modified glass bead analogs

Methods: Thiol modified surfaces were prepared by immersing gold sputter-coated glass coverslips (10.5 x 20mm and 10.5 x 3mm), in 1mM concentration of thiol in ethanol solutions for 24 hours. Prior to thiol deposition, the gold-coated coverslips were immersed in a 0.125% solution of butyltrichlorosilane (BTS) in chloroform for 15 min. This was done to block any glass that might have been exposed due to minor scratches occurring during sample preparation. Coverslips treated with dodecanethiol (C12), served as the model hydrophobic surface and coverslips treated with 11-mercaptoundecanoic acid (11-MUA) served as model anionic hydrophilic surfaces. Octadecyltrichlorosilane (OTS) modified and plasma cleaned glass beads served as the thiol surfaces hydrophobic and hydrophilic analogs, respectively. Horizontal, static contact angles of prepared surfaces were measured (Table 1). XPS was utilized to verify the sample surface's thiol composition. These samples were then used in in vitro coagulation assays using 50% blood plasma in phosphate buffered saline.

Table 1: Horizontal static contact angles of surfaces.

Surface	*Static Contact Angle (mean ± std. dev.)*
11-mercaptoundecanoic acid	35 ± 1°
Glass	< 10°
Dodecanethiol	108 ± 1°
Octadecyltrichlorosilane	103 ± 1°

Results: The model hydrophobic and anionic hydrophilic surfaces performed similarly to their silane-modified glass bead analogs. Figure 1displays the clotting times of the thiol modified surfaces in the coagulation assays. As expected, the hydrophilic surface clotting much faster than the hydrophobic surface. Clotting time studies performed with the 10.5 x 3mm thiol-modified coverslips produced clotting times elevated as compared to clotting times performed with the 10.5 x 20mm thiol-modified coverslips. This dependence on the amount of surface area present is consistent with the mathematical model developed previously using silane-modified glass beads.

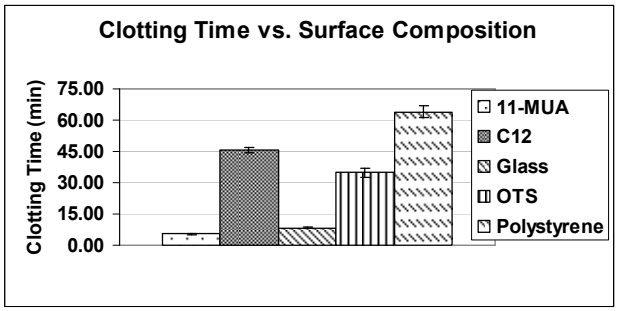

Figure1: Clotting times of 10.5 x 20mm thiol surfaces and silane bead analogs of comparable surface area.

Summary: In this first step, the thiol-modified surfaces were shown the elicit plasma coagulation responses similar to their modified glass bead analogs. Plasma coagulation response to positively-charged thiol and mixed-thiol surfaces will be investigated as the next step in the process to create surfaces with nanoscale patterning.

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