Contact activation of coagulation by mixed thiol self-assembled monolayers

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Introduction: Coagulation resulting from contact activation in blood-material interactions remains a challenge in the use of blood-contacting cardiovascular materials. The widespread view of contact activation imparts coagulation factor XII (FXII) activating properties 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], and more recently have shown decreased levels of activation for surfaces with mid-level wetting (~40-60º contact angle) [2]. Further, FXIIa generation in plasma is found to be attenuated at hydrophobic surfaces rather than accentuated at hydrophilic surfaces [1,3], thus the need to further explore the role of the surface in contact activation.

Statement of Purpose: In this study, one- and two-component thiol-modified surfaces were prepared and utilized as the procoagulant for in vitro coagulation assays and FXII activation in neat buffer solution.

Methods: Mixed carboxyl/methyl- and hydroxyl/methyl-thiol modified surfaces were prepared by immersing gold-coated coverslips (10.5 x 20mm) in 1mM of thiol in ethanol solutions for 24 hours. Dodecanethiol, 11-mercaptoundecanoic acid, and 11-mercapto-1-undecanol were obtained from Sigma and used as received. Prior to thiol deposition, the gold-coated coverslips were immersed in a 0.125% solution of butyltrichlorosilane (BTS) in chloroform for 15 min to block any glass exposed due to minor scratches/edges. XPS was utilized to verify the sample surface's thiol composition and estimate the proportion of the individual components. These samples were then used in in vitro coagulation assays using a 50% mixture of recalcified plasma in phosphate buffered saline. Alternatively, the samples were placed into purified FXII solutions for 30 minutes, with or without an HSA displacement step after 25 min. [FXIIa]eq amounts were calculated using the methods described in [4]. Briefly, an FXIIa titration curve is prepared from the same lot of plasma. From this curve, parameters describing activation and propagation of the cascade are derived, and allows for calculation of FXIIa produced by surface contact.

Results: Figure 1 shows [FXIIa]eq for mixed hydroxyl/methyl-surfaces in the plasma coagulation assay, and illustrates a notable decrease in FXIIa produced by materials having adhesion tensions in the range of 40-50 dyne/cm. For mixed carboxyl-/methyl- surfaces there is a sigmoidal transition region between 45-60 dyne/cm with samples with adhesion tensions <45 dyne/cm having near 10-fold larger [FXIIa]eq than their mixed hydroxyl analogs (data not shown). When assaying FXII activation in neat buffer solutions, results indicate that for both carboxyl-/methyl- and hydroxyl-/methyl surfaces with adhesion tensions near 45 dyne/cm, activation is decreased (Figure 2) and that FXII activation by the hydrophilic one-component hydroxyl- and carboxyl-terminated surfaces were statistically indistinguishable.

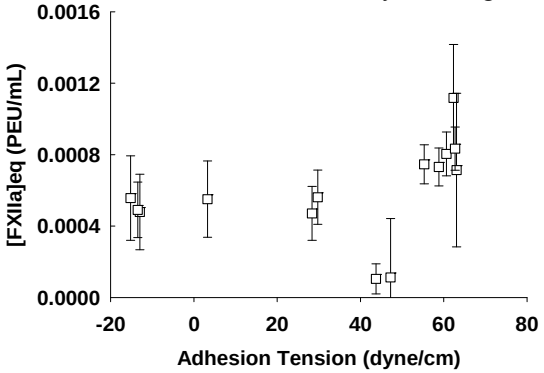

Figure 1: Plasma coagulation assay results for mixed hydroxyl surfaces (mean ± SD; n=3). Surfaces varied from pure methyl- (left) to pure hydroxyl-terminated (right).

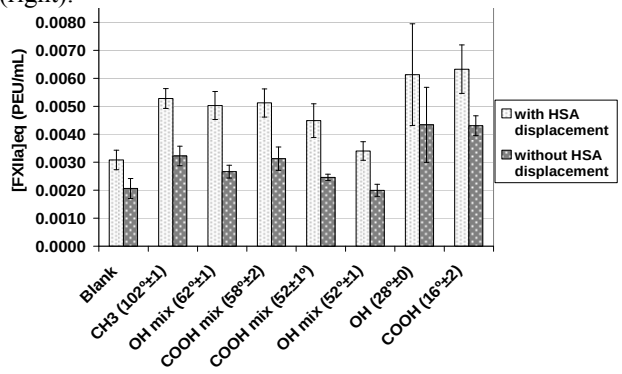

Figure 2: FXII activation (mean ± SD; n ≥ 6) in neat buffer solution. Surface types are listed along with their contact angle ± SD.

Summary: While plasma coagulation studies support a role for anionic surfaces in contact activation, the results suggest FXII activation in neat buffer solution is related to surface wettability. A minimum is seen for materials with τ = 45-60 dyne/cm, and there is no statistically distinguishable difference between pure COOH and OH surfaces. Future studies include the investigation of mixed amine-/methyl-terminated monolayers and the effect of nanoscale patterning on plasma coagulation and FXII activation.

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