Activation of FXII in plasma at biomaterial interfaces

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Statement of Purpose: Thrombosis persists as a common problem associated with blood-contacting medical devices. Full understanding of the molecular basis of hemocompatibility is essential to the development of advanced cardiovascular biomaterials. Recent data showing that FXII activation in neat buffer solution is not specific to hydrophilic surfaces [1] stands in sharp contrast to the traditional biochemistry suggesting that activation is specific to negatively-charged surfaces [2]. These new findings further show that FXII activation in the presence of plasma proteins leads to *an apparent specificity* for hydrophilic surfaces that is actually due to a relative diminution of the FXII→FXIIa reaction at hydrophobic surfaces. To further probe details of surface activation, this study compares FXII activation in plasma by the three known activation reactions — autoactivation (activation by FXII binding to an activating surface), self-amplification (FXII activation by FXIIa, also termed autohydrolysis), and reciprocal-activation (mediated by Kallikrein) — using silanized-glass test surfaces and a previously-developed model of the intrinsic cascade [3].

Methods: Three test solid procoagulants in order of decreasing hydrophilicity: clean glass particles (0.5 mm Ø), 3-aminopropyltrichlorosilane modified (APS) and octadecyltrichlorosilane modified (OTS) glass particles, were prepared as described previously [3]. Prekallikrein (PK)-deficient platelet-poor-plasma (dPPP) was prepared from PK-deficient plasma obtained commercially (George King Biomedical, Overland, KS) [3]. PPP was prepared by reconstituting dPPP (RdPPP) with 40 μg/mL PK (Enzyme Research Lab, South Bend, IN). The coagulation response to exogenous FXIIa (Enzyme Research Lab) and the test surfaces was measured using an in vitro coagulation assay [3] in dPPP and RdPPP. Previously-published models used here were based on the premise that solid procoagulant generates FXIIa, which is processed by a "gray box" containing all intermediate steps of the cascade to ultimately generate a fibrin clot.

Results/Discussion: FXIIa titration of dPPP and RdPPP were fit to a model to generate parameters a, b and c, that describe the processing of a FXIIa dose by the gray box to form a clot (measured as coagulation time, CT). Surface area titration (SAT) plots (Fig. 1) in dPPP and RdPPP were fit to the model to determine the "catalytic efficiency" at each surface. Using the gray box model the amount of FXIIa generated for a given CT was calculated from the SAT plots [3]. Previous work demonstrated that FXII self-amplification at material surfaces is insignificant in plasma [3]. Calculations from fitting SAT data in RdPPP yielded total enzyme produced via the remaining two pathways, while calculations from data in dPPP yielded the amount of FXIIa generated via auto-activation. Differences between these values yielded FXIIa production via reciprocal-activation. The calculated amounts of FXIIa generated for each test surface at three select surface areas via the reciprocal- and auto-activation pathways (Fig. 2) indicate that FXIIa generated in total and along each pathway scales with water-wettability.

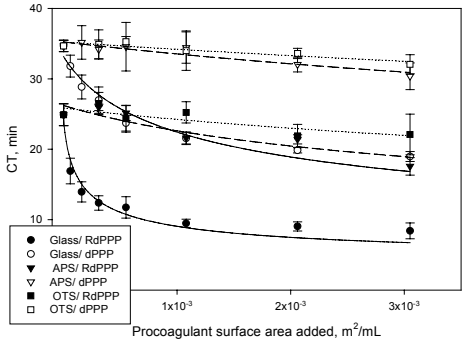

Fig.1: SAT plots of test surfaces- glass, APS and OTS- in dPPP and RdPPP. Lines are the best fit of a coagulation model .

Further, the fraction of FXIIa generated by reciprocal-activation for a given test surface (Table 1) indicates that this pathway is the largest component of FXIIa generation at all biomaterial surfaces though the amounts scale with water-wettability. Although the large error associated with the data arising from propagation of errors through the model precludes drawing a statistically significant conclusion, trends in the data are indicative of a modest increase in the fraction of FXIIa contributed by reciprocal-activation with increasing water-wettability.

Table 1: Fractions generated by reciprocal-activation at each surface

Surface	[reciprocal-FXII] /[total-FXIIa] (%)
Glass	~ 90
APS	~ 82
OTS	~ 75

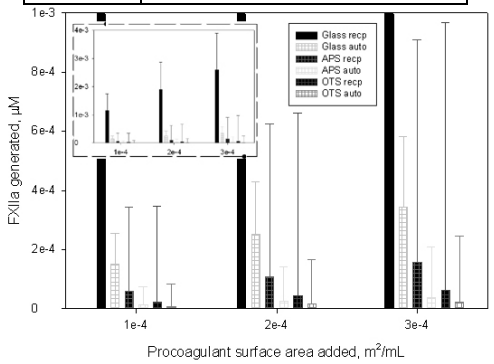

Fig.2: Calculated amounts of FXIIa generated by auto and reciprocal activation until coagulation at three select surface areas for each for the three test surfaces

Conclusions: Amounts of FXIIa generated in plasma at test activating surfaces spanning a broad surface-energy range by autoactivation, autohydrolysis, and reciprocal activation were calculated. PK-mediated reciprocal-activation was shown to be the principal activation pathway whereas autohydrolysis was insignificant. Amount of FXIIa produced by each pathway scaled with activating surface energy.

References: 1. Zhuo R, et al, Biomaterials 2006. **27**: 4325
2. Kaplan AP, Prog Hemost Thromb. 1978. **4**: 127
3. Guo Z, et al, Biomaterials 2006. **27**: 796

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