FXIIa Products Produced by Surface Contact Activation

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Statement of Purpose: Plasma coagulation is influenced by the surface properties of blood-contacting materials. Experimental evidence suggests that plasma coagulation is proportional to the extent of endogenous FXII activation, which produces a bolus of FXIIa that is dependent on surface energy and surface area[1-2]. Understanding the details of FXII autoactivation in buffer solution allows for closer examination of FXII/surface interactions, and thus benefits engineering biomaterials with improved hemocompatibility. This study focused on investigating the properties of the activation products produced in response to 3 different activators having different surface properties.

Methods: Glass particles in 425-600 µm diameters (Sigma Aldrich) were sequentially cleaned using aquaregia and piranha solutions, and then thoroughly rinsed with DI water. After drying, one portion of clean glass particles was used as a Type II (hydrophilic) activator, while the remaining beads were modified with octadecyltrichlorosilane (OTS) as a Type I activator or 3-aminopropyltrichlorosilane (APTES) as a type 0 activator[3]. FXII autoactivation in PBS buffer was carried out in dynamic mode as outlined below: 500 µL of 30 µg/ml FXII in PBS was brought into contact with activators from $1.0x10^{-5}$ to $5.0x10^{-3}$ m^2/mL for 1 hr on a rotating hematology mixer. The products present in the supernatant were then assessed by a plasma coagulation time assay for procoagulant yields, and a chromogenic assay by cleaving the commercial substrate Pefa-5963 for amidolytic yields.

Results / Discussion:

Surface-area dependence of FXII autoactivation in plasma and buffer (Figure 1). For clean glass, both the surface area titration (SAT) in plasma and buffer are surface area dependent, showing asymptotically decreasing coagulation time (CT) to a plateau, with increasing surface area up to 2.5 x 10^{-3} m^2/mL. By contrast, no significant differences in CT were noted with increasing surface area for any other materials in buffer.

FXII autoactivation in buffer is surface energy and surface area dependent (Figure 2). Procoagulant yields rose up from 9.1 x 10^{-3} PEU/ml to 5.9 x 10^{-1} PEU/ml when the surface area of clean glass increased from 1.0 x 10^{-3} m^2/mL to 5.0 x 10^{-3} m^2/ml. By contrast, increasing surface area of OTS or APTES did not enhance the generation of procoagulant products, with statistically equivalent yields of ~ 10^{-5} PEU/ml at OTS and 10^{-6} PEU/ml at APTES. At a given surface area, the procoagulant yield is surface energy dependent scaling exponentially in the order: APTES<OTS<clean glass. For a given material, amidolytic activity was statistically similar for all surface areas. Table 1 shows that fractional yield of procoagulant fragments (P/A) is surface energy dependent, with highest P/A at clean glass, followed by OTS and APTES. Increasing surface area of clean glass resulted in higher relative yield of procoagulants, suggesting that hydrophilic surfaces tend to produce fragments that exhibit a greater proportion of procoagulant than amidolytic activity.

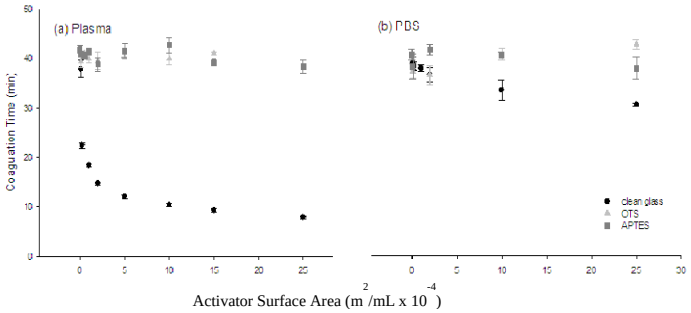

Figure 1. Comparison of surface area titration by different activators in (a) plasma and (b) PBS buffer

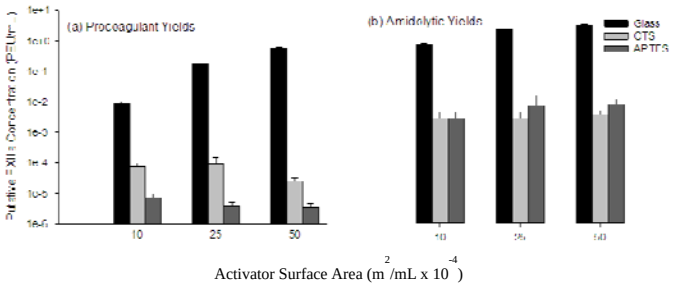

Figure 2.Yield of procoagulant (a) and amidolytic (b) protein fragments as a function of activator surface area by of FXII autoactivation in PBS buffer solution with different activators.

Table 1. Summary of FXII autoactivation products induced by activators of varying surface energy and surface areas.

Particles	Surface Area (m^2/mL x 10^{-4})	Procoagulant activity (PEU/ml)	Amidolytic activity (PEU/ml)	Ratio (procoagulant/ amidolytic)	Error of propagation
Glass	10	9.06E-03	8.46E-02	0.11	9.96E-03
	25	1.81E-01	2.23E-01	0.81	3.73E-02
	50	5.86E-01	2.98E-01	1.96	1.32E-01
OTS	10	7.80E-05	7.90E-04	0.099	4.69E-02
	25	8.94E-05	7.90E-04	0.11	8.82E-02
	50	2.47E-05	9.88E-04	0.025	1.17E-02
APTES	10	7.16E-06	7.90E-04	0.0091	5.06E-03
	25	4.03E-06	1.78E-03	0.0023	2.06E-03
	50	3.51E-06	1.98E-03	0.0018	8.80E-04

Conclusions: FXII contact activation in buffer produces activation products having both procoagulant and amidolytic activities. The ratios of these products are dependent on surface type, with hydrophilic clean glass producing a higher proportion of procoagulant to amidolytic products than the other materials.

Reference:

1. Chattejee et al., Journal of Biomedical Materials Research Part A, 2009, 90A, 27
2. Golas et al. , Biomaterials, 2010, 31, 1068
3. Golas et al., Biomaterials, 2011, 32, 9747