

The Effects of Activated Non-Aggregated Platelets on Blood Plasma Coagulation

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Statement of Purpose: Thrombus induced by biomaterials is partially due to platelet-mediated reactions and partially due to coagulation of blood plasma itself. Both depend on the surface properties of implanted materials and characteristics of the blood flow. Much of what we know about the blood coagulation cascade involves blood-plasma coagulation *in vitro* using plasma that is depleted of platelets. This approach simplifies the blood coagulation problem, but the role of platelet, especially when activated; in contribution to blood coagulation is not clear. This study isolated activated non-aggregated platelets and measured the coagulation time of plasma in the presence of platelets with other activators to investigate the relative contribution of platelets and activators on coagulation.

Methods: Glass beads were rigorously cleaned in aquaregia and piranha solutions, respectively, and rinsed with copious amount of DI water. After drying, one portion of clean glass beads was used as model hydrophilic surface, while the portion modified with octadecyltrichlorosilane (OTS) to create hydrophobic surfaces. The beads were used as activators in a plasma coagulation assay. Human platelet rich plasma (PRP) was separated from healthy donor blood, and platelets were isolated through Sepharose 4b column using PBS effluent. To prevent platelet aggregation, RGDS peptide was added to block the binding sites of $\alpha IIb\beta 3$ receptor on the platelet membrane. Platelets were activated by addition of ADP. Platelet aggregation was measured by a Chrono-Log Aggregometer. The isolated activated non-aggregated platelets were used with activators in the *in vitro* coagulation assay measuring coagulation time (CT) which is described elsewhere¹⁻³.

Results / Discussion:

Isolation of activated non-aggregated platelets. Isolated platelets produced by the Sepharose column did not show ADP or Collagen induced aggregation. The platelets were supplemented with fibrinogen and $CaCl_2$ solution, but similar results were obtained. However, supplementing isolated platelets back into PPP led to aggregation (Fig. 1). Various RGDS peptide and platelet concentrations were examined by the aggregometer with $75 \times 10^3/\mu L$ platelets. Results showed that $50 \mu M$ of peptide produced the largest knockdown in aggregation.

Surface area titration of human plasma. Without platelets, coagulation time of plasma in response to bead activators depends on surface properties and area. A significant decrease in CT was observed with the hydrophilic glass surfaces compared to hydrophobic, especially at low surface area, while very slight changes in CT occurred on the hydrophobic surfaces until the surface area exceeded 2000 mm^2 , and no change in CT was observed with area larger than 2000 mm^2 .

Effect of activated non-aggregated platelets on plasma coagulation. Without activators (glass beads or FXIIa), the activated non-aggregated platelets dramatically reduced the

CT of plasma (fig 3 control), demonstrating activated platelets are a cofactor in coagulation. Significant changes in CT were observed among the samples having different numbers of platelets when activated by hydrophobic beads. The addition of hydrophilic glass beads further decreased CT, however, and no significant changes in CT were observed between samples with different concentration of platelets. Similar results were observed in the case of activation by enzyme FXIIa.

In conclusion, platelets have a dramatic effect on CT of plasma in the presence of weak activators (the control tube and hydrophobic beads) compared to strong activators (hydrophilic beads and enzymes).

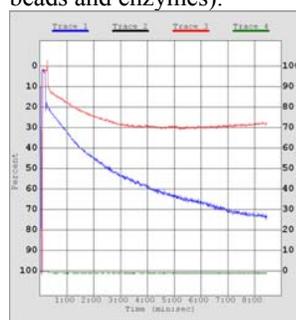


Figure 1. Aggregation of isolated platelets added back into PPP. Red line represents peptide-bound platelets (29% aggregation) while the blue is unbound platelets (72% aggregation)

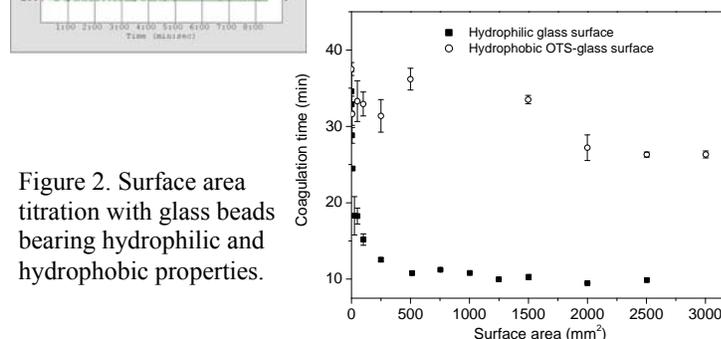


Figure 2. Surface area titration with glass beads bearing hydrophilic and hydrophobic properties.

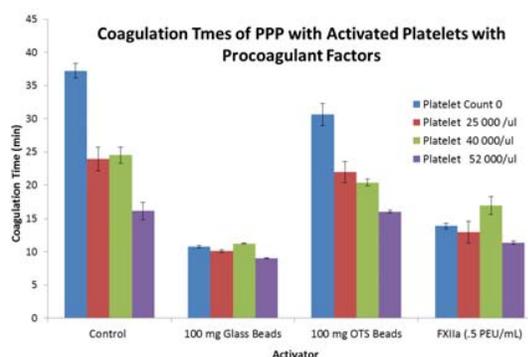


Figure 3. Coagulation time of plasma in the presence of activated platelets and activators.

Reference:

1. Chattejee et al., Biomaterials, 2006, 27, 5643
2. Zhuo et al., Biomaterials, 2006, 27, 4325
3. Chattejee et al., Biomaterials, 2009, 30, 4915