An FVIII-derived Peptide Enables VWF-binding of an Artificial Platelet Substitute without Interfering with Natural Platelet Adhesion to VWF

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Statement of Purpose: There is significant clinical interest in artificial platelet substitutes for potential applications in transfusion medicine. To this end, our research is focused on peptide-lipid nanoconstructs that integrate platelet-inspired key hemostatic functions of *injury site-selective adhesion* and *site-directed active platelet aggregation promotion.* We have recently demonstrated that these functionally integrated constructs promote enhanced hemostatic activity *in vitro* as well as *in vivo* [1-3]. For *injury site-selective adhesion*, we have utilized a FVIII-derived VWF-binding peptide (VBP) [4]. FVIII binds to D'-D3 domain while natural platelet GPIbα binds to A1 domain of VWF. Therefore, we hypothesized that our VBP-decorated nanoconstructs will adhere to VWF without interfering with natural platelets binding to the same VWF. Here, we report on experimentally testing this hypothesis and also studying whether the adherent VBP-decorated constructs can enhance platelet aggregation when co-decorated with a fibrinogen-mimetic peptide (FMP).

Methods: *Platelet binding to VWF:* VWF was adsorbed onto glass coverslips then treated with ristocetin to facilitate conformational exposure of platelet-binding A1domains. This VWF-surface was incubated with calcein (green fluorescence)-stained platelets. In a control experiment, platelet binding to VWF was blocked by pre-incubation of the VWF-surface with soluble glycocalicin, which is the extracellular domain of GPIbα that binds to the A1 domain of VWF. As additional controls, platelets were incubated with VWF without ristocetin or on a BSA non-adhesive surface. Platelet binding to the surfaces was imaged using fluorescence microscopy and quantified by surface averaged intensity analysis.

VBP-decorated construct binding to VWF: VWF was adsorbed onto glass coverslips and treated with ristocetin. The VWF-surface was exposed to incubation with rhodamine-labeled (red fluorescence) VBP-decorated constructs. In comparison studies, the VWF was pre-incubated with A1-blocking glycocalicin before incubation with VBP-decorated constructs. As additional controls, VBP-decorated constructs were incubated without ristocetin or a BSA non-adhesive surface. Construct binding was quantified by fluorescence intensity analysis.

Simultaneous binding of constructs and platelets to VWF: For these studies, the ristocetin-treated, VWF-adsorbed surfaces were exposed to incubation with calcein-stained (green) platelets and rhodamine-labeled (red) VBP-decorated constructs, simultaneously. For comparison, VWF surfaces were co-incubated with platelets and undecorated constructs. To study whether co-decoration of VBP-decorated constructs with FMP amplifies platelet *aggregation* onto the VWF surface, VBP-FMP-constructs (red) were simultaneously incubated with platelets (green) on VWF-adsorbed ristocetin-treated surfaces, in absence or presence of ADP. The co-localization of red and green fluorescence (yellow) indicated construct-mediated platelet aggregation onto the surfaces and this was quantified by platelet fluorescence intensity analysis.

Thrombin cleavage of VBP: Physiologically, VWF-bound FVIII is cleaved by thrombin to release FVIIIa fragment. Therefore, we tested the stability of our FVIII-derived VBP construct binding to VWF in the presence of thrombin by exposing the red fluorescent construct-adhered surfaces to thrombin incubation and analyzing post-exposure fluorescence intensity.

Results: VBP-decoration of constructs resulted in significant adhesion to ristocetin-treated VWF, even if the A1-domain was blocked by glycocalicin. To compare, A1-blocking resulted in significant reduction of platelet adhesion. Moreover, the VWF-adhesion of constructs was unaffected by thrombin. Without A1-blocking, the VBP-decorated constructs and natural platelets could adhere to ristocetin-treated VWF simultaneously without mutual interference. Furthermore, the VBP-FMP-co-decorated constructs enhanced aggregation of platelets on the VWF surface, especially in presence of ADP (**Figure 1**).

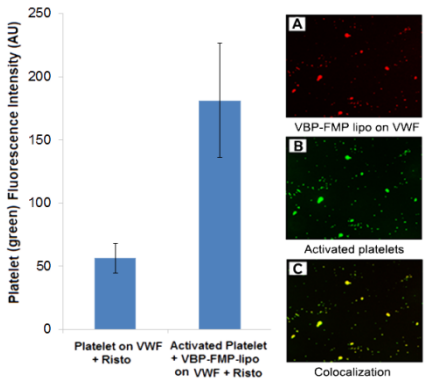

Figure 1. VBP-FMP constructs enhance platelet aggregation on VWF surface.

Conclusion: Decoration with the FVIII-derived VBP can render stable VWF-adhesion of artificial platelet constructs by a mechanism distinct from platelet GPIbα interaction with VWF's A1 domain. This adhesion is possibly through VWF's D'-D3 domain.

References:

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