The control of protein binding on bio-interface by a novel coating to prevent contact activation on biomaterials

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Abstract

The coagulation system activates quickly to "protect" the body when most biomaterials come in contact. To prevent thrombotic risk, thus administration of anticoagulants is necessary but it comes with the bleeding risk. So, research efforts are focused intensely on endowing the material with anti-thrombogenic properties. A consensus has been reached that bio-interface-induced contact activation (BCA) is the principal pathway for thrombogenesis induced by biomaterials. Inhibiting FXII-surface interaction (protein-resistant surface) seems to be the only guideline currently available [1,2]. We provide an alternate approach by manipulating the FXII-surface interaction.

Background

Under normal physiological conditions, the intrinsic coagulation pathway initiated via the contact activation system plays a major role in surface-induced thrombosis. The initial protein that triggers such activation is FXII which can adsorb to foreign surfaces to generate FXIIa with the assistance of PK. FXIIa can further cleave PK to form kallikrein, whereupon kallikrein can then back activate FXII to generate more FXIIa, resulting in a positive feedback loop. FXIIa can also cleave and activate FXI to generate FXIa and thus trigger activation of FIX, leading to downstream generation of FXa and thrombin (**Fig. 1**).

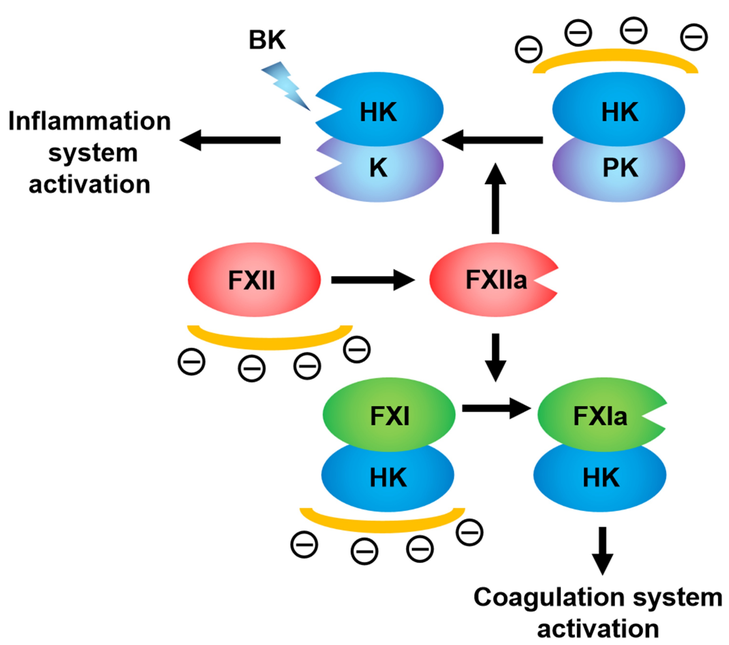

Figure 1.

Schematic diagram for blood-contacting material-induced contact activation. Surface binds FXII and promotes its autoactivation to FXIIa. HK promotes FXI activation by FXIIa, and the resulting FXIa then propagates coagulation leading to thrombin generation. FXIIa also activates HK-bound prekallikrein (PK) to kallikrein (K), which activates FXII in a reciprocal manner to promote additional FXIIa generation. Release of bradykinin from HK cleaved by K induces an inflammatory response.

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Query! Whether protein-resistant surface can prevent the induced coagulation

By avoiding the adsorption of proteins on the material surface, intuitively, it may prevent the coagulation activation induced by the material. However, in reality, the influence of the material on proteins is not limited to the interface on the material surface. We found that after interaction between coagulation factor FXII and glass or other antifouling surface, activation occurs and diffuses back into the bulk solution (**Fig. 2**).

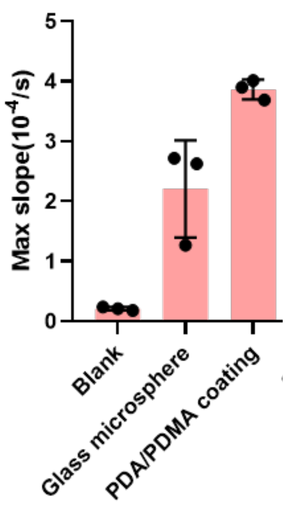

Figure 2.

Cleavage efficiency of S-2302 by an in-vitro simulated contact initiation system (400 nM of FXII and 400 nM of PK in PBS buffer) after incubation with glass and PDMA coated glass microparticles. The slope can represent the catalytic activity of the incubated contact initiation system. At least n = 3 biologically independent samples, all values are expressed as the mean ± s.d.

Selective Protein Interaction Coating (SPIC) can prevent the induced coagulation

The developed coating can be applied to diverse substrates without the need of pre-treatment. The coating stabilizes FXII on the surface and prevents contact activation thereby preventing thrombogenesis while maintaining homeostasis. (**Fig. 3**).

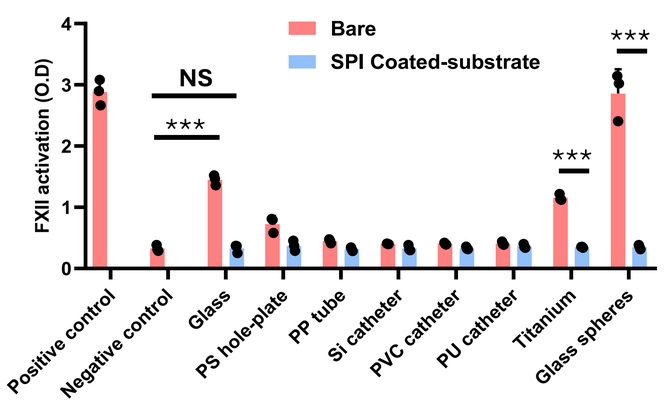

Figure 3.

FXII stabilizing coating can prevent surface-induced contact activation and thrombin generation without interfering with hemostasis balance. (a-b) The designed coating can prevent contact activation induced by diverse substrates. At least n = 3 biologically independent analyses were performed. ***p < 0.001, NS represents p > 0.05 comparing with negative control. All values are expressed as the mean ± s.d.

Animal experiments

An arteriovenous shunt model in rabbit is developed to demonstrate the potential of the new coatings. The bare catheter induced severe intra-catheter thrombosis and the blood flow through the catheter was almost stopped, while the SPI coated catheter significantly reduced surface induced-thrombosis (**Fig. 4**).

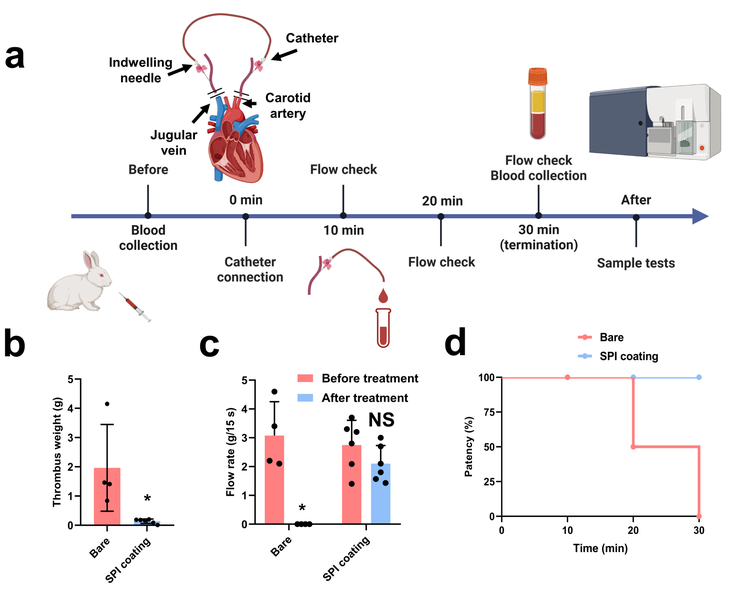

Figure 4.

FXII stabilizing coating can inhibit material-induced coagulation activation in an ex-vivo arteriovenous shunt model without anticoagulant administration. (a) Schematic representation of the animal experiment, (b-d) the prevention of thrombus generation induced by catheters. At least n = 3 biologically independent analyses were performed. *p < 0.05, NS represents p > 0.05 comparing with negative control. All values are expressed as the mean ± s.d.

Conclusions and outlook

1. Coagulation proteins, especially FXII, are generally adsorbed onto almost all surfaces. **We found that surface-FXII interaction may not be a sufficient condition for the activation of FXII.**

2. Conventional protein repelling surfaces cannot completely prevent surface-protein interactions. There is no clear understanding of surface-FXII interactions and the consequent conformational changes of FXII by these surfaces, **thus it is difficult to suggest anti-fouling/protein resistant surfaces are antithrombotic** [3,4].

3. Understanding how different **FXII binding sites** [5] affect its **conformational changes** and which conformations of FXII are prone to the coagulation activation will shape our future development.

References

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