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# Bioengineered surfaces to improve the blood compatibility of biomaterials through direct thrombin inactivation ☆

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ABSTRACT

Thrombus formation, due to thrombin generation, is a major problem affecting blood-contacting medical devices. This work aimed to develop a new strategy to improve the hemocompatibility of such devices by the immobilization of a naturally occurring thrombin inhibitor into a nanostructured surface. Boophilin, a direct thrombin inhibitor from the cattle tick *Rhipicephalus microplus*, was produced as a recombinant protein in *Pichia pastoris*. Boophilin was biotinylated and immobilized on biotin-terminated self-assembled monolayers (SAM) via neutravidin. In order to maintain its proteinase inhibitory capacity after surface immobilization, boophilin was biotinylated after the formation of a boophilin–thrombin complex to minimize the biotinylation of the residues involved in thrombin–boophilin interaction. The extent of boophilin biotinylation was determined using matrix-assisted laser desorption/ionization-time of flight/time of flight mass spectrometry. Boophilin immobilization and thrombin adsorption were quantified using quartz crystal microbalance with dissipation. Thrombin competitive adsorption from human serum was assessed using $^{125}$I-thrombin. Thrombin inhibition and plasma clotting time were determined using spectrophotometric techniques. Boophilin-coated SAM were able to promote thrombin adsorption in a selective way, inhibiting most of its activity and delaying plasma coagulation in comparison with boophilin-free surfaces, demonstrating boophilin's potential to improve the hemocompatibility of biomaterials used in the production of blood-contacting devices.



## 1. Introduction

Thrombus formation is still one of the major complications affecting blood-contacting medical devices [1]. Blood clotting induced by the surface of these devices is due to a complex series of highly interlinked events, starting with the adsorption of plasma proteins, followed by the activation of complement and coagulation systems and by the adhesion and activation of platelets and leukocytes [2]. Initial protein adsorption is dependent on the chemical and physical properties of the biomaterial surface [1]. One strategy to increase the hemocompatibility of these devices could be the surface immobilization of antithrombotic agents, such as thrombin inhibitors [3], since this enzyme plays a central role in the coagulation system. Thrombin is produced at the end of the coagulation cascade and is responsible for converting fibrinogen to fibrin. After polymerization, fibrin is crosslinked and stabilized into an insoluble gel by the thrombin-activated factor XIIIa. Thrombin is also able to stimulate platelet activation and to promote its own production through activation of factors V, VIII and XI [4]. In addition to these procoagulant activities, thrombin plays important roles in inflammation [5] and in the activation of complement factors [6].

The indirect thrombin inhibitor heparin has been used to coat the surface of several biomaterials [7,8]. However, heparin-coated surfaces have severe limitations. They only inhibit thrombin after binding of endogenous antithrombin (AT), which limits the therapeutic use of heparin-coated surfaces in patients with low amounts of AT (e.g., in some septic situations) [9,10]. Other disadvantages include the inability of the heparin–AT complex to inhibit enzymatically active and procoagulant fibrin-bound thrombin and the scavenging of heparin by platelet factor 4 released by activated platelets [11,12].

☆ No benefit of any kind will be received either directly or indirectly by the authors.

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