Layer-by-Layer Assembly with Combined Nitric Oxide Generation and Surface Immobilized Heparin -- A Universal Anti-Thrombotic Coating for Biomedical Implants

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Statement of Purpose: Local delivery of anti-platelet and/or anticoagulant reagents at the blood-device interface has been widely studied to suppress thrombogenic events that are associated with implants. Nitric oxide (NO), a natural platelet inhibitor released by a healthy endothelium, is of specific interest due to its natural occurrence and highly localized effect. It has been reported that NO can be catalytically generated from NO carriers in the blood such as *S*-nitrosothiols (RSNOs) via the use of polymer coatings with immobilized Cu(II) or organoselenium (RSe) sites. One such coating was fabricated by a so-called Layer-by-Layer (LbL) process in which anionic sodium alginate (Alg) and cationic polyethyleneimine pre-modified with RSe catalyst were alternately adsorbed on a pre-charged surface via electrostatic attraction.[1]

Heparin is an anticoagulant reagent commonly administered to patients during and after cardiovascular intervention to prevent thrombosis. It catalyzes the inhibition of thrombin by antithrombin III (AT III) and thereby impedes the coagulation cascade. Herein we describe a new antithrombotic LbL coating that combines NO generation capability and surface immobilized heparin activity.

Methods: Heparin (sodium salt) from porcine mucosa and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma-Aldrich (St. Louis, MO). Human AT III and bovine Factor Xa were purchased from Haematologic Technologies Inc. (Essex Junction, VT). Bovine serum albumin (BSA) solution was obtained from Invitrogen (Carlsbad, CA). Chromogenic substrate S-2222 was a generous gift from Instrumentation Laboratory Inc. (Lexington, MA). The RSe immobilized LbL was prepared and tested for NO generation activity as previously described,[1] except that the LbL has SePEI as the outmost layer instead of Alg. Heparin was then immobilized on such an amine functionalized LbL surface via various means, including electrostatic attraction (A), in the chain immobilization via amide bond (B) and end-attachment via formation of Schiff base (C). In method A, heparin was attracted on the surface using a similar mechanism as employed to prepare the LbL coating, using 2 or 4 cycles with SePEI as polycation. In method B, heparin was allowed to react with equivalent molar EDC first to activate its carboxylate groups. In method C, heparin was partially fractionated by nitrous acid as reported[2] to create aldehyde end groups. Subsequently, the amine LbL surface was immersed in the solution of modified heparin (B and C) to react for 12 h. The bioactivity of surface immobilized heparin was determined by a chromogenic anti-FXa assay.

Results: The bioactivity of surface immobilized heparin is inversely correlated to the absorbance of the assay at 405 nm which is caused by the highly selective thrombogenic reaction between FXa and S-2222. As shown in Fig. 1, all three methods resulted in FXa inhibitory effect. However, the surface reacted with EDC activated heparin (method B) shows the highest heparin activity. According to the calibration curved obtained using the same assay configuration, the surface heparin activity of B was quantified to be 8.2±0.4 mU/cm^2.

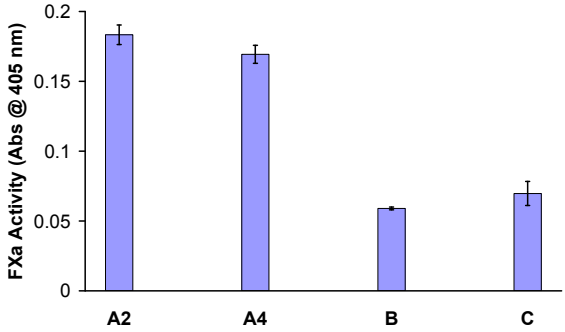

Figure 1. Surface heparin activities of NO generating surfaces modified with: A2) two (Hep/SePEI) bilayers; A4) four (Hep/SePEI) bilayers; B) EDC activated and C) partially fractionated heparin.

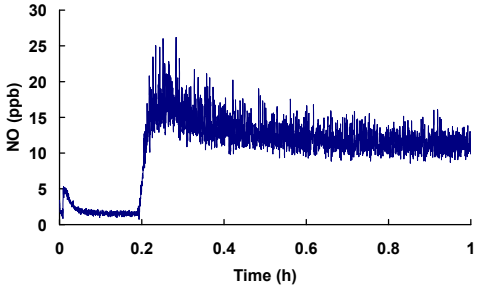

Figure 2. NO generation by LbL modified with heparin via method (B) from 50 μM GSNO and 50 μM GSH.

The NO generation activity of the LbL coating was also confirmed after modification. As shown in Fig. 2, exposure of LbL treated with method B to RSNO solution yields significant NO flux from the surface as measured by chemiluminescence detection.

Conclusions: Various means have been explored to immobilize heparin on an NO generating LbL surface. Among these, heparin immobilized via amide bond formation shows the highest anti-FXa activity and the resulting coating retains significant NO generation activity. Since some electrostatically adsorbed heparin on the surface may slowly release upon exposure to blood, future studies will focus on evaluating heparin activity after prolonged exposure to plasma.

References:

1. Yang J.;Langmuir 2008:24:10265-10272.
2. Larm O.; Biomater. Med. Devices Artif. Organs 1983:11:161-173