Catalytic Nitric Oxide Generation via Layer-by-Layer Assembly on Metal Surfaces

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Statement of Purpose: Acute thrombotic complication is a common adverse event associated with stent facilitated angioplasty. It is now acknowledged that this thrombogenic response can be greatly reduced by releasing or generating low levels of nitric oxide (NO), a potent anti-platelet agent, at the device-blood interface. Recently, we reported a layer-by-layer (LbL) assembly constructed by manually submerging a substrate into solutions containing sodium alginate (Alg) and polyethylenimine possessing covalently linked organoselenium (RSe) species (SePEI), in an alternating dip coating fashion to create up to 15 bilayers.[1] The RSe sites on the PEI are capable of catalytically generating NO from *S*-nitrosothiols (RSNO), a class of endogenous NO storage compounds present in the blood. However, a physiologically relevant level of NO production was not achieved due to the relatively limited amount of RSe sites in the very thin LbL structure produced by manual deposition of the film. Here, we report on the automated preparation of NO generation LbLs with as many as 100 bilayers on metals typically used to fabricate stents (i.e. stainless steel, titanium, and nitinol) to generate a physiologically relevant level of NO under typical biological conditions.

Methods: Polyethyleneimine (PEI, M_w 25 kD) and sodium alginate (Alg, M_w 12-80 kD) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. *S*-Nitrosoglutathione (GSNO) and organoselenium immobilized polyethyleneimine (SePEI) were synthesized as previously described.[1] Metal strips were briefly washed in acetone, ethanol, and deionized water shortly before the LbL assembly process. The LbL was coated by alternately dipping the metals in 1 mg mL^{-1} SePEI and Alg solutions (10 min each) with three intermediate PBS washings (1 min each) to remove loosely attached species prior to deposition of the next counter polyion layer. Such a coating cycle was repeated until the desired number of bilayers was reached. A homemade automated LbL deposition system was employed to ensure consistency of the deposition cycles across various samples.

Results: The successful construction of the multilayer structure was proved by detecting the Se content within the LbL. As shown in Fig. 1, the elemental Se content within the multilayer displayed a linear correlation with the number of bilayers. Meanwhile, the Se concentrations in the LbLs are substrate nonspecific, except for $(SePEI/Alg)_{100}$ coated on NiTi which slightly deviated from the Ti and SS data. The catalytic activity of the LbL coated on metal substrates was first evaluated using excess GSNO and GSH to better elucidate the trend. It revealed that the LbLs coated on different metal substrates possessed similar NO generation capability (see Fig. 2). With 50 µM GSNO and 50 µM GSH, the LbLs yielded a considerable NO production upon immersion in the reaction solution. When the catalytic multilayer was removed from the reaction reservoir, the NO generation almost completely ceased. The slight baseline increase indicated predominantly heterogeneous NO production within the multilayer structure. With 1 µM GSNO and 20 µM GSH, the LbLs produced a NO flux of ca. 0.6 x 10^{-10} mol cm^{-2} min^{-1}, which is relevant to the biological NO flux from the EC monolayer as estimated to be 0.5 - 4 x 10^{-10} mol cm^{-2} min^{-1}.[2]

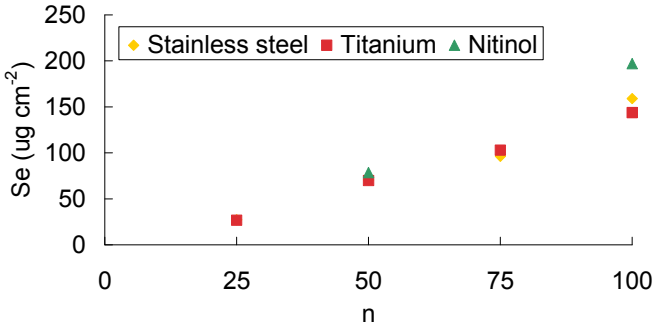

Figure 1. Se density within $(SePEI/Alg)_n$ coated on Stainless Steel, Ti, and Ntinol.

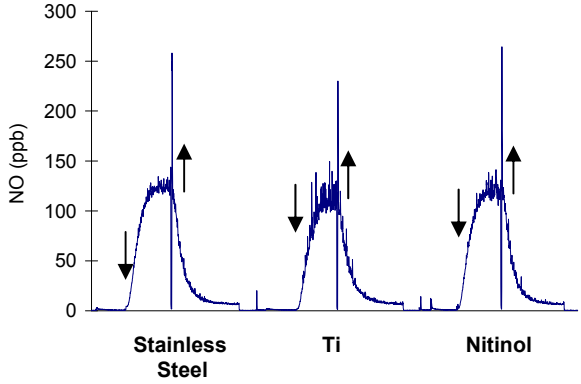

Figure 2. NO generation by $(SePEI/Alg)_{25}$ coated on SS, Ti and NiTi from 50 µM GSNO, 50 µM GSH. The metal pieces were immersed into/removed from the reaction cell as indicated by the (↑) and (↓) arrows.

Conclusions: A previously reported NO generating LbL coating was translated onto metal substrates. Stainless steel, nitinol and titanium were selected due to their dominating use in coronary stents. A consecutive deposition of SePEI and Alg polyelectrolytes was realized on these metal surfaces by monitoring the selenium quantity during the coating procedure. The resulting LbLs fabricated on metal substrates showed substantial structural stability and catalytic activity.

References:

1. Yang J.;Langmuir 2008:24:10265-10272.
2. Vaughn, MW J. C. *Am. J. Physiol. Heart Circ. Physiol.* 1998, 43, H2163-H2176.