Carboxyl-Ebselen-Based Layer-by-Layer Coating for Nitric Oxide Generation

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Statement of Purpose: Nitric Oxide (NO) is a potent anti-platelet agent that can prevent thrombus formation on medical devices. Endothelial cells, with an NO flux of $0.5\sim4\times10^{-10}$ mol cm^{-2} min^{-1}, exhibit anti-thrombotic activity.[1] NO generation materials are a group of catalytic coatings that are capable of generating NO from endogenous NO donors in blood, such as *S*-nitrosothiols (RSNOs). Recently, NO generation materials based on organoselenium species, e.g., selenocystamine, have been developed.[2] However, the *in vivo* toxicity of alkyldiselenides greatly limited their potential biomedical application. Aromatic selenium species are known to be less toxic than their aliphatic counterparts. Ebselen (see Fig. 1), for example, is undergoing phase III clinic trial in Japan due to its low toxicity, anti-inflammatory and anti-oxidation properties.[3] Here, we report the first effort to use an ebselen-based layer-by-layer (LbL) film for NO generation. The LbL coating is capable of generating biological levels of NO from RSNOs and is expected to widen the application of Se-based NO generation materials due to reduced toxicity.

Methods: Carboxyl-ebselen (C-ebselen) (see Fig. 1) was synthesized using a reported method.[4] Polyethyleneimine (PEI, M_w 25 kD) and sodium alginate (Alg, M_w 12-80 kD) were purchased from Sigma-Aldrich. Carboxyl-ebselen immobilized polyethyleneimine (e-PEI) was synthesized by EDC/NHS coupling of 36.9 mg carboxyl-ebselen with 200mg polyethyleneimine in 20 ml pH 6.0 MES buffer (molar ratio -COOH:EDC:NHS=1:6:4). Glass and quartz slides were cleaned with piranha solution (7:3 H_2SO_4/30%H_2O_2) and the LbL films were prepared by alternately dipping the substrates in 1 mg mL^{-1} e-PEI and Alg solutions (10 min each) with three intermediate PBS washings (1 min each) using a homemade automated deposition system. The NO generated was detected by purging N_2 into the solution and monitored using a chemiluminescence NO analyzer (NOA) (Sievers 280, Boulder, CO).

Results: The initially prepared LbL films are cloudy (Figure 1A, insert) and not stable. Significant leaching of catalyst species is observed in the NOA experiments for the film without annealing.

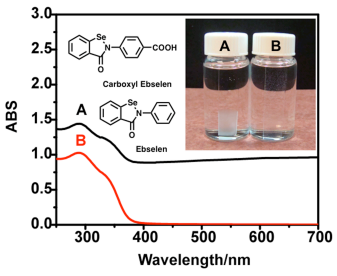

Figure 1. UV-vis spectra of (e-PEI/Alg)$_{50}$ on a quartz slide A) before and B) after salt annealing,. Insert shows the appearance of (e-PEI/Alg)$_{50}$ on glass slides A) without annealing and B) after salt annealing

The large background signal observed in the UV spectra from 400 nm to 700 nm (Fig. 1A) is due to the scattering of light of the heterogeneous film. When placed in PBS containing 1.5M NaCl, the film turned from cloudy to clear within 1 min (Figure 1B, insert). The background absorbance went back to zero (Figure 1B), indicating the formation of a homogeneous film. The films were further cross-linked with EDC/NHS in PBS containing 1.5 M NaCl and soaked in PBS solution containing 100 µM EDTA and 50 µM GSH to reduce all the catalytic sites into selenolates and remove trace interferences.

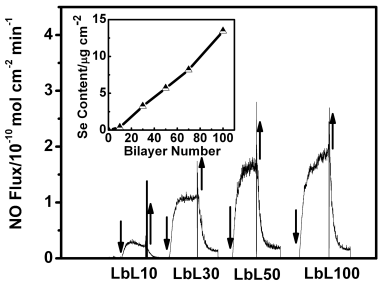

Figure 2. NO Flux vs layer numbers of annealed (e-PEI/Alg)$_n$, the films are inserted and taken out of the NOA cell containing 100 µM EDTA, 50 µM GSH, 50 µM GSNO in 2ml PBS pH=7.4 as indicated by the ↓ and ↑ arrows. Insert shows the increase of Se content with number of bilayers in the LbL films

Figure 2 shows the NO generation behavior of such LbL films in the presence of *S*-nitrosogluthathione (GSNO) and glutathione (GSH). When a film was inserted into the NOA cell, a stable NO flux was observed. The signal went back to almost baseline when the film was removed, indicating low leaching of catalytic sites from the LbL films. As shown in Figure 2, the NO flux of the annealed LbL films increases with increased layer numbers, but the growth slows down significantly after 50 bilayers. In contrast, the Se content of the LbL flms grows exponentially with layer numbers (Figure 2, insert). The inconsistency might come from the increased difficulties in mass transfer when a thick film was obtained at later stage of LbL growth. An annealed (e-PEI/Alg)$_{100}$ was able to generate NO from 100 µM EDTA, 5 µM GSH, 5 µM GSNO at 37°C. The NO flux is 0.55×10^{-10} mol cm^{-2} min^{-1}, which is relevant to physiological release rates from healthy endothelial cells.[1]

Conclusions: An aromatic selenium species with low *in vivo* toxicity has been incorporated into a LbL film for NO generation. The LbL film is able to generate NO from RSNOs and is a potentially novel anti-thrombotic coating.

References:

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