

Layer-by-Layer Assembly for Nitric Oxide Generation Based on Catalytic Decomposition of S-Nitrosothiols by Organoselenium Species

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Statement of Purpose: Nitric Oxide (NO) is a well-known contributor to the thromboresistant nature of a healthy endothelium due to its ability to inhibit platelet adhesion and activation. Polymeric coatings with functional moieties capable of catalytically generating NO from endogenous S-nitrosothiol species (RSNO) have attracted considerable interest for their potential as thromboresistant coatings on biomedical devices. Recently, several organoselenium species (RSe) were reported to be catalytically active in generating NO from RSNOs when immobilized on cellulose filter paper and dialysis membranes.¹ However, a more generic coating with immobilized RSe catalysts that is applicable for use on a wide variety of surfaces is still needed. Layer-by-layer (LbL) deposition is a newly developed technique to deposit multilayer composites by the attraction between oppositely charged polyions.² It is simple to process such coatings making them attractive for creating stable NO generating surfaces on medical devices. Most importantly, the properties of these films are largely independent on the nature of the substrate. Here, we report results from our initial studies on the use of the LbL technique with RSe species covalently linked to polycationic polyethyleneimine and polyanionic heparin for catalytic NO generation.

Methods: Polyethyleneimine (PEI, Mw 25k), polydiallyldimethylammonium chloride (PDDA, Mw 100k-200k), heparin (Mw 17k-19k), glutathione (GSH), were obtained from Sigma-Aldrich (St. Louis, MO) and used as received. The RSe attached PEI (SePEI) and S-nitrosoglutathione (GSNO) were synthesized as described previously.¹ The LbL coating was prepared by dipping a cleaned quartz substrate alternately into SePEI (or PDDA) and heparin solutions (1 mg/ml each) for 10 minutes. Briefly, two PDDA/heparin bilayers were first coated onto the substrate as a precursor layer to stabilize the surface charge. Then, SePEI and heparin were coated for a given number of cycles. To monitor the stepwise growth of the multilayers, the SePEI was labeled with fluorescein-5-isothiocyanate (FITC) and the coating was characterized using UV-Vis spectrophotometer (Lambda 35, Perkin Elmer, MA) after every 2nd coating cycle. To assess catalytic activity, the coated slides were inserted into a GSNO (50 μ M) solution containing EDTA (0.5 mM), along with GSH as reducing agent (100 μ M). The NO generated was purged from the test solution with N₂ flow and detected using a chemiluminescence NO analyzer (NOA) (Seivers 280, Boulder, CO).

Results/Discussion: As more LbL coating cycles are performed, the UV-Vis spectra display progressively greater absorbance at 500 nm due to the FITC chromophore, indicating an increasing amount of SePEI being deposited on the substrate (Fig. 1).

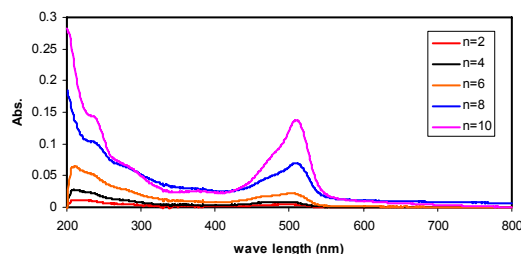


Figure 1. UV-Vis of LbL coating (SePEI/heparin)_n (n is the number of coating cycles performed).

After inserting the coated slide into the GSNO/GSH test solution, a large initial burst of NO flux is observed which decreases rapidly until a steady state is reached (see Fig. 2). This steady state NO production exists for an extended time period but ceases upon removal of the LbL coated slide from the solution. Reinserting the slide into the solution replicates the observed steady state NO production. Also, no remarkable baseline increase is observed during the NOA experiments, ruling out the possible leaching of the catalyst from the coating into the solution (Fig. 2).

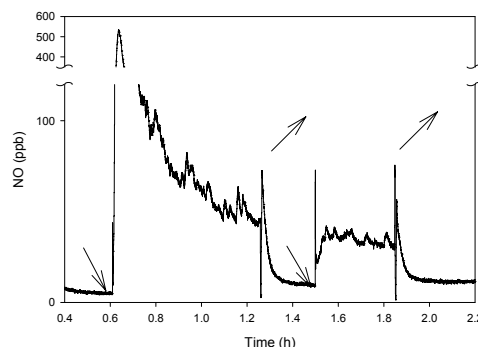


Figure 2. NOA of (SePEI/heparin)₁₅ in deionized H₂O containing 50 μ M GSNO, 100 μ M GSH and 0.5 mM EDTA. Note that the given slide is inserted into (↓) and removed from (↑) the solution at each arrow point indicated.

Conclusions: An LbL film was successfully prepared using organoselenium species linked PEI and heparin as the polyions. Preliminary NOA testing indicates that such a coating can catalytically decompose GSNO to NO in the presence of a reducing agent (GSH). Future work will focus on crosslinking such a multilayer structure to strengthen its stability in buffer solution.

References:

1. Cha W. Biomaterials. 2007;28:19-27.
2. Decher G. Science. 1997;277:1232-1237