DNA Immobilization on Surfaces via Mussel Adhesive Protein-Inspired Polymer

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Statement of Purpose: DNA microarray performance depends strongly on surface chemistry and the method used for DNA probe immobilization[1]. While numerous strategies for immobilizing oligonucleotide capture probes onto surfaces have been reported, they often require material-specific surface 'priming', the conditions of which must be specially tuned for each substrate material. We previously developed catechol and amine containing small molecule and synthetic polymer mimics of mussel adhesive proteins (MAPs), which proved to be effective in forming coatings on a variety of materials[2,3]. Here, we report a new mussel-mimetic catecholamine polymer for DNA microarray preparation. The facile approach allows for microarray preparation on a variety of substrates, including noble metals, ceramics, semiconductors, and synthetic polymers.

Methods: The mussel-mimetic random copolymer p(DOMA-EAMA) was synthesized by free radical polymerization of N-(3,4-dihydroxyphenethyl) methacrylamide (DOMA) and aminoethyl methacrylamide (EAMA) monomers. The polymer had a catechol content of 10.6 wt% (UV/vis) and molecular weight of 210 kDa (GPC). Substrate materials (Au, Pt, poly(styrene) (PS) and poly(methylmethacrylate) (PMMA)) were prepared via sputtering (Au, Pt) or spin-coating (PS, PMMA) onto DNA microarray glass slides. p(DOMA-EAMA)-coated surfaces were formed by immersion of different substrates for 24 hrs in 1mg/ml p(DOMA-EAMA) in 10mM Tris, pH 8.3. Amine-terminated single-stranded capture probes were manually spotted on p(DOMA-EAMA)–coated substrates using conventional spotting buffer and hybridization performed in standard hybridization buffer. Surfaces were analyzed by X-ray photoelectron spectroscopy (XPS) and hybridization detected using fluorescent capture probes.

Results: XPS was performed to detect p(DOMA-EAMA) adsorption onto Si wafer and binding of the DNA on the polymer-coated surface (Figure 1). Bare Si (bottom) mainly exhibited strong characteristic substrate signals (Si2p @ 98.5 eV and O1s @ 532.0 eV). Polymer-coated Si sample (middle) displayed a decrease in the Si signal and an increase in the C1s (284.5 eV) and N1s (399.5 eV) signal, demonstrating successful surface modification by p(DOMA-EAMA). After DNA immobilization onto polymer-coated substrates, a P2p (134.0 eV) was observed.

Capture probes were spotted onto these surfaces and hybridization was tested via Cy5-labeled target probes (Figure 2). Fluorescence was observed only after spotting capture probe onto p(DOMA-EAMA)-coated surfaces and hybridizing with a sequence-matched target probe. Low background fluorescence was observed.

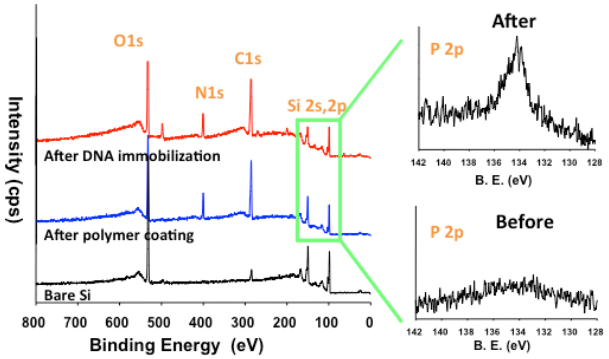

Figure 1. XPS survey spectra of bare, p(DOMA-EAMA)-coated, and DNA capture probe immobilized Si wafer. Inset: High-resolution spectra of the P_{2p} region of polymer-coated surface before (bottom) and after (top) immobilization of DNA capture probe.

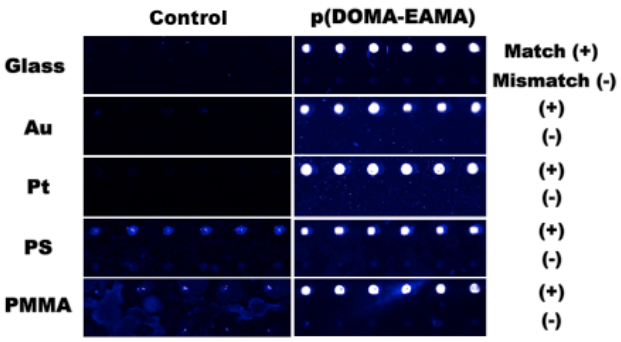

Figure 2. DNA hybridization on uncoated (left) and p(DOMA-EAMA)-coated (right) substrates spotted with capture probe. Fluorescence images of 2×6 oligonucleotide microarray on five different substrates after hybridization with immobilized amine-modified match or mismatch capture probes.

Conclusions: We demonstrated a simple surface modification strategy for DNA microarray fabrication using a catecholamine polymer. An easy and chemically mild one-step immersion of the substrates in a polymer solution formed a thin film on noble metals, oxides and polymer substrates. This strategy may simplify the preparation of DNA microarrays, potentially broadens the range of substrate materials for microarray fabrication, and should be useful for immobilization of cDNA, peptides, or aptamers.

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References: 1. Dawson ED. Anal. Biochem. 2005; 341: 352-360. 2. Lee H, et al. Science. 2007; 318: 426-430. 3. Lee H, et al. Adv. Materials. 2008; 20: 1619-1623.