DNA Immobilization

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Facile DNA Immobilization on Surfaces through a Catecholamine Polymer**

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Numerous strategies for biomolecular detection and analysis rely on simple, robust and cost-effective methods for immobilizing DNA, proteins, and other biomolecules onto surfaces. In most cases the methodology employed for biomolecule immobilization is closely linked to performance, with a key feature being the choice of linking chemistry. A number of strategies have been reported and include physisorption, covalent coupling, and biospecific interactions (e.g. avidin–biotin). However, strategies developed for one substrate/biomolecule pair often prove to be ineffective with others due to subtle changes in substrate chemistry, or require extensive system-specific optimization. As a result, there is a continuing need to identify new and versatile surface modification approaches that avoid such biomolecule- and substrate-specific effects.[1]

A good illustration of these challenges is given by the immobilization of oligonucleotides, DNA, and RNA on surfaces, frequently performed for genetic material diagnostics,[2] therapeutics,[3] military,[4] environmental,[5] and consumer technologies.[6] For DNA microarrays that are now widely used in diagnostics, performance depends strongly on substrate surface chemistry and the method used for oligonucleotide probe immobilization.[7] Numerous methods exist for linking DNA onto surfaces through covalent[8,9] and noncovalent interactions.[10] The choice of immobilization method is strongly driven by considerations of the substrate surface chemistry and highly specific protocols are developed for each substrate material. For example, silica substrates routinely used in DNA microarray technology are functionalized with silane coupling agents and further reacted with a variety of secondary polymers or cross-linking reagents to provide covalent coupling of DNA.[9,11] However, silane coupling agents do not typically work well on noble metals. Instead direct immobilization of thiol-modified DNA molecules to the metal surface through metal–sulfur bonds is a more effective approach.[12,13] Indirect metal attachment through a functional thiol-SAM is also a common strategy.[14] For example, Forch and co-workers have developed a sophisticated DNA sensor based on plasma polymerization of allylamine on thiol-SAM modified Au.[14] Polycations have also been used to immobilize DNA through electrostatic interactions, however they are limited to charged substrates and may require additional surface activation steps for robust attachment.[15]

DNA immobilization on polymer surfaces can be more challenging and has been previously attempted by methods such as layer-by-layer polyelectrolyte assembly,[16] atom transfer radical polymerization (ATRP) of block copolymers,[17] activated agarose film coating,[18] and spin-coating of end-functional diblock copolymers.[19] For example, Chen et al. have demonstrated DNA immobilization on glass, silicon wafers, and poly(methyl methacrylate) (PMMA) by spin-coating an alkyne end-functional block copolymer for surface "click" reactions.[19] Plasma polymerization can in theory be applied to various substrates but it can be technically demanding and substrate selection can still be significant.[14,20] As a result, no broadly applicable approach to polymer substrate surface modification with DNA has been demonstrated, and there is a continuing need to identify simple and versatile approaches which avoid substrate-specific effects during linking of biomolecules to surfaces and aggressive "priming" surface treatments or activation.[1]

Here, we describe a new mussel-mimetic catecholamine polymer that strongly adsorbs to a variety of substrates and binds DNA molecules without altering its biological activity. We illustrate the method by spotting oligonucleotides onto noble metals, metal oxides, semiconductors, and synthetic polymer substrates coated with the catecholamine polymer. The approach employs simple immersion in mild aqueous solutions, and is demonstrated by hybridization of bound DNA with a complimentary oligonucleotide sequence in a manner reminiscent of DNA microarray analysis.

We previously developed a range of synthetic polymer and small-molecule mimics of catechol- and amine-rich

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mussel adhesive proteins (MAPs).[21] Mussel-inspired polymers are effective in forming adherent coatings on a variety of materials and can be exploited to confer a range of properties to substrates, serving as a platform or "primer" for further functional modification to yield thin metal films, pseudo-self-assembled monolayers, antifouling grafted polymer films, and layer-by-layer assemblies. Here, a mussel-mimetic random copolymer p(DOMA-AEMA) (Figure 1a) was synthesized

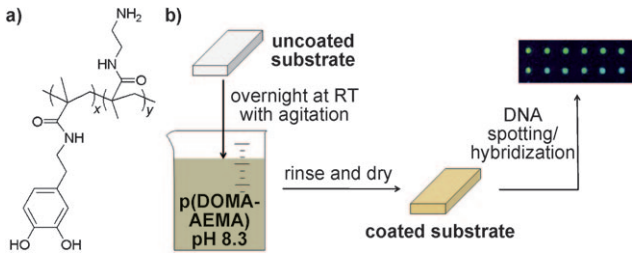

Figure 1. a) Chemical structure of p(DOMA-AEMA), a synthetic catecholamine polymer mimic of mussel-adhesive protein. b) Three-step method for preparing DNA microarray on substrates: 1) immersion of p(DOMA-AEMA) in an alkaline solution to create a thin polymer film; 2) rinsing and drying; 3) spotting the substrates with DNA in a microarray format.

by free radical polymerization (see Supporting Information) of *N*-(3,4-dihydroxyphenethyl) methacrylamide (DOMA) and aminoethylmethacrylamide (AEMA) monomers. p(DOMA-AEMA) was designed to contain key chemical constituents present at high concentration in mussel adhesive proteins found near the plaque–substrate interface.[22,23] For example, the amino acids 3,4-dihydroxyphenylalanine (DOPA) and lysine (Lys) together represent over 50% of the total amino acids found in Mefp5, a prominent MAP.[23] Catechol groups form coordination bonds on inorganic surfaces; or they may oxidize into reactive quinones or semiquinones under oxidative conditions, subsequently forming strong irreversible covalent bonds on organic surfaces,[24] or giving rise to intermolecular cross-linking of the polymers. Amine groups may also contribute to mussel adhesion through electrostatic interactions and hydrogen bonding. Accordingly, p(DOMA-AEMA) was designed to include the catechol and amine functional groups found respectively in the side chains of DOPA and Lys residues. The resulting polymer had a catechol content of 10.6 wt% (UV/Vis) and molecular weight in the range 160–210 kDa (GPC) (Supporting Information). Au, Pt, poly(styrene) (PS), and PMMA substrates were prepared by sputtering (Au, Pt) or spin-coating (PS, PMMA) on standard glass microscope slides. p(DOMA-AEMA)-coated surfaces were formed by immersion of substrates for 24 h in 1 mg mL^{-1} p(DOMA-AEMA) in 10 mM Tris buffer at pH 8.3. Amine-terminated single-stranded capture probes were manually spotted on p(DOMA-AEMA)-coated substrates using conventional spotting buffer, and hybridization was performed in a standard hybridization buffer (Figure 1). Surfaces were analyzed by X-ray photoelectron spectroscopy (XPS) and contact angle measurements, and hybridization was detected using fluorescent target analyte.

Silicon wafer was first used as a model substrate to study p(DOMA-AEMA) adsorption and binding of DNA on the p(DOMA-AEMA)-coated Si (Figure 2). Adsorption of

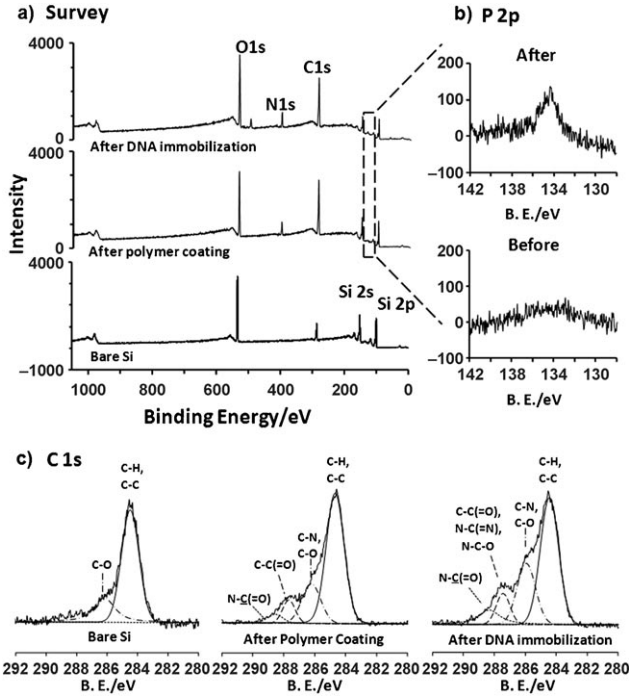

Figure 2. XPS characterization of p(DOMA-AEMA)- and DNA-coated substrates. a) XPS survey spectra of bare, p(DOMA-AEMA)-coated, and capture probe DNA-immobilized Si wafer (100 μM, 2 h). b) High-resolution P 2p region of polymer-coated surface before and after immobilization of DNA capture probe. c) C 1s region after each modification step.

p(DOMA-AEMA) to Si wafer (Figure 2a, middle) resulted in a decrease in the Si signal and an increase in the C 1s (284.5 eV) and N 1s (399.5 eV) signals compared to the bare Si, demonstrating successful surface modification by p(DOMA-AEMA). The continued presence of the Si signal after p(DOMA-AEMA) modification indicates that the thickness of the polymer film was less than the escape depth of photoelectrons (ca. 10 nm).[25] This was further confirmed by spectroscopic ellipsometry, which revealed an approximate polymer thickness of 2.4 nm (Supporting Information, Figure S2). As a sensitive surface analytical tool, XPS has been used for detailed studies of DNA interfacial chemistry on surfaces.[12,26,27] After DNA immobilization onto p(DOMA-AEMA)-coated Si, a distinct P 2p (134.0 eV) peak was observed, whereas virtually no P 2p peak was detected on p(DOMA-AEMA)-coated Si (Figure 2b). Figure 2c shows the high-resolution C 1s XPS spectra for each modification step. Emergence of peaks at 287.5 eV and 288.5 eV and increased peak intensity at 286.2 eV correspond to the polymer coating on Si wafer (Figure 2c, middle). A C 1s high-resolution spectrum of the DNA-immobilized surface showed further increased intensity at 286.2 eV, 287.5 eV, and 288.5 eV (Figure 2c, right). Especially, the peaks at 287.5 eV and 288.5 eV represent carbon species specific to the DNA

bases,[26] confirming the binding of oligonucleotides on polymer coated surface.

Surface chemical composition calculated from high-resolution XPS spectra are shown in Table S3. The Si content decreased from 50.3% to 21.1% after modification with p(DOMA-AEMA), whereas significant increases in N (0.1 to 7.3%) and C (14.4 to 49.6%) contents were observed after coating Si wafer with p(DOMA-AEMA). After DNA immobilization, Si, N, C, and O content changed only slightly compared to p(DOMA-AEMA)-coated substrates. Measurable phosphorous was detected only on the DNA-immobilized substrates. Consistent with the XPS observations, static water contact angle changes also confirmed the sequential formation of polymer coating and DNA binding. Increased hydrophobicity was measured after polymer coating (49° to 54°), which decreased slightly to 50° after DNA immobilization (Table S3).

The amines and catechols of p(DOMA-AEMA) confer a wide range of potential chemical interactions with substrates. For example, in the case of Si substrates, adsorption is likely mediated by electrostatic interactions between protonated amine groups of the polymer and the negatively charged native oxide of Si, as well as possibly through bidentate charge-transfer complexes formed between catecholic OH groups in catechol and the native oxide surface.[24,25] With respect to organic substrates, catechols are further capable of covalent and strong noncovalent interactions,[24,28] as well as π-electron, hydrogen-bonding, and other interactions with substrates. Thus, we surmised that the chemical bonding versatility afforded by the presence of catechols and amines in the polymer may confer upon p(DOMA-AEMA) the chemical attributes necessary to interact strongly with many inorganic and organic substrates. Consistent with this notion, similar XPS results were obtained for adsorption of p(DOMA-AEMA) on Au, Pt, glass, PS, and PMMA substrates (Figure S4). Static water contact angle was about (53 ± 3)° on polymer coated surfaces independent of the substrate. Representative XPS survey scans and water droplet images obtained during contact angle measurements are shown in Figure S4 and S5 and Table S4.

To demonstrate the use of p(DOMA-AEMA) to mediate DNA immobilization, amine-terminated capture oligonucleotide probes (Oligo 1: complementary, Oligo 2: noncomplementary to target analyte) were spotted onto p(DOMA-AEMA) coated substrates, and hybridization was tested with Cy5-labeled target analyte (Oligo 3) using standard DNA microarray methodology (Figure 3 and S5). Intense fluorescence spots indicative of hybridization were observed on coated substrates after hybridization with a sequence-matched target analyte. The spot intensity and morphology were relatively consistent within the array sets on the various substrates studied (Figure S5). This could imply the ability of p(DOMA-AEMA) to bind probe DNA regardless of the underlying substrate. Further optimization of spotting techniques and polymer coating conditions may lead to improved performance across various substrates. There was consistently very low fluorescence on noncomplementary capture probe spots, indicating that the sequence specificity of target analyte binding was preserved on p(DOMA-AEMA)-coated surfaces. In the absence of p(DOMA-AEMA) coating, unmodified glass, Au, and Pt exhibited no fluorescence of capture probe spots and virtually no background fluorescence, suggesting little nonspecific interaction of capture and probe DNA with the substrate surface. Unmodified PS and PMMA substrates exhibited detectable but low levels of spot fluorescence after hybridization with sequence matched target analyte.

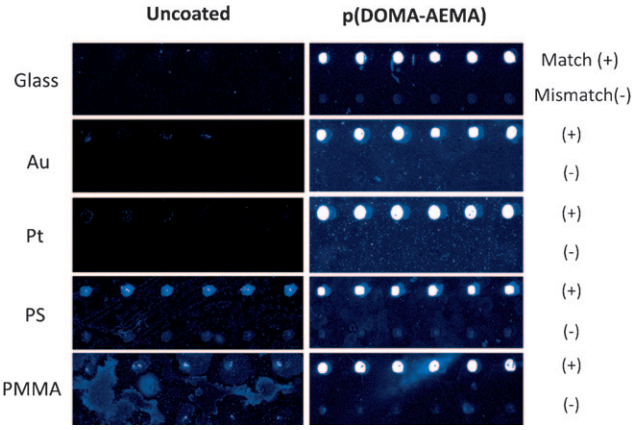

Figure 3. Fluorescence images of DNA hybridization on uncoated and p(DOMA-AEMA)-coated substrates spotted with capture probe (20 μM) in a 2×6 microarray pattern on five different substrates. In each case, spots were made using amine-modified capture probes that were matched (Oligo 1, top rows) or mismatched (Oligo 2, bottom rows) with the fluorescent target analyte.

The performance of DNA microarray fabricated on p(DOMA-AEMA)-coated glass slides was further characterized by comparing hybridization sensitivity at different capture probe spotting concentrations (1 to 100 μM), and at varying target analyte concentrations (100 pM to 1 μM). A representative image of hybridization on p(DOMA-AEMA) film is shown in Figure 4a, which was obtained after hybridization with 1 nM of target analyte on spotted capture probes. A quantitative analysis of hybridization efficiencies at different capture and target concentrations was performed. As shown in Figure 4, hybridization signal intensities decreased at lower capture probe concentrations (1 to 20 μM), indicating the effects of immobilized capture probe density on hybridization efficiency. XPS measurements of the P 2p region also qualitatively showed the expected correlation in intensity based on the probe DNA spotting concentration and target hybridization (Figure S3). For probe spotting concentrations >2 μM, the detection limit of target concentration on p(DOMA-AEMA)-coated surface was between 100 pM and 1 nM with a signal to background ratio (S/B) of 4.7 ± 0.6 (Figure 4b and Figure S7). From these results, we conclude that the DNA microarray fabricated on p(DOMA-AEMA) film has wide dynamic range (ca. 4 orders of magnitude) and low detection limit (0.1–1 nM).

It is interesting to note the following additional features of catecholamine polymer mediated DNA immobilization, which may be considered advantageous in comparison to other immobilization strategies. First, a blocking step is unnecessary to achieve the results reported. A prehybridization or blocking reagent, which includes bovine serum

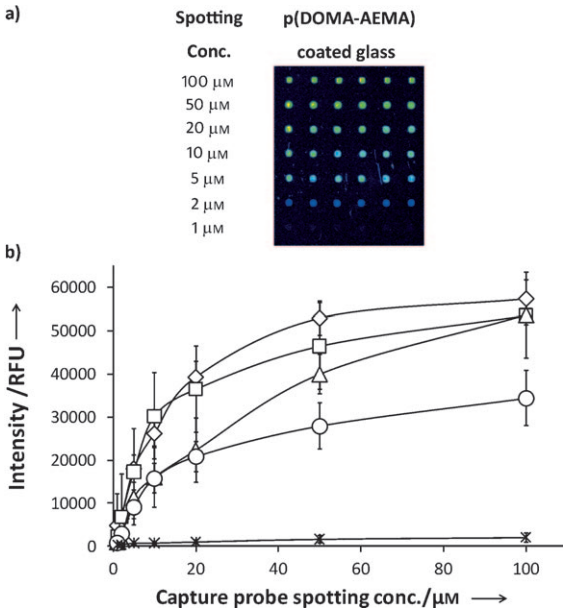

Figure 4. Representative hybridization results on p(DOMA-AEMA) coated glass slides. a) Amine-modified capture probe (Oligo 1) was spotted at different concentrations (1–100 μM) and hybridized with Cy5-labeled target analyte (Oligo 3, 1 nM). b) Capture probe (Oligo 1, 1–100 μM) was spotted on polymer-coated substrates and hybridized with target analyte (Oligo 3, 100 pM–1 μM; *: 100 pM, ○: 1 nM, △: 10 nM, □: 100 nM, ◇: 1 μM). Fluorescence intensities are averaged values calculated by subtracting background intensities from spot intensities of multiple spots. Error bars represent ±1 SD of at least 24 replicates. RFU = relative fluorescence units.

albumin or ethanolamine to prevent nonspecific binding of target analyte, is often employed in DNA microarray protocols.[29] However, the blocking step was not critical in this study as Figure 3 and Figure 4 were generated without any explicit efforts to prevent nonspecific binding of target analytes except for the addition of surfactants normally present in DNA buffer solutions[30] and careful washing after capture probe binding.[31] Second, vigorous substrate precleaning was not required. The results shown were obtained on substrates prepared simply by sonication in isopropyl alcohol prior to polymer coating. We tentatively attribute this feature to the p(DOMA-AEMA) catechol and amine moieties, which are known to confer on mussel adhesive proteins the ability to bind to unclean surfaces. Simpler substrate requirements may translate to less costly protocols for DNA microarray manufacturing. Finally, we note that in addition to amine-modified capture probes, thiol- and unfunctionalized capture probes were also successfully treated (Figure S8). This may be advantageous in that the immobilization of unfunctionalized probe DNA onto p(DOMA-AEMA) would eliminate the time-consuming and costly need for probe functionalization.

The mechanism for DNA probe immobilization by p(DOMA-AEMA) is likely due to multiple catechol and amine interactions that may be covalent or noncovalent in nature.[24] For example, free catechols within the p(DOMA-AEMA) coating not bound to the substrate may become oxidized to quinones and subsequently react with the terminal amines[24,32] and thiols[33] of the capture probe DNA. Noncovalent interactions with p(DOMA-AEMA) are also likely to play a role in DNA binding. These may take the form of hydrogen-bonding and π-electron interactions as well as electrostatic interactions between the p(DOMA-AEMA) amines and the DNA phosphate backbone. Physisorption of DNA on p(DOMA-AEMA) polymer with a heat treatment after probe spotting is also possible. A schematic of the proposed binding mechanism is shown in Figure S9.

In conclusion, we demonstrated a simple surface modification strategy for DNA immobilization using a new catecholamine mussel-mimetic polymer. In particular, 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 that allowed immobilization of DNA strands without further surface activation or treatment. This strategy potentially broadens the range of substrate materials that can be used for the preparation of DNA microarrays as well as simplifies their preparation. We also anticipate that this strategy will be useful for the immobilization of different types of biomolecular probes, such as cDNA, peptides, aptamers, or direct polymerase chain reaction (PCR) products.

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