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Fabrication and Surface Characterization of DNA Microarrays Using Amine- and Thiol-Terminated Oligonucleotide Probes†

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A versatile chemistry utilizing the homobifunctional cross-linker 1,4-phenylene diisothiocyanate (PDC) to attach both amine- and thiol-terminated oligonucleotides to aminosilane-coated slides was examined in a microarray format. Three common aminosilanes, 3-aminopropyltriethoxysilane (APS), *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane, and *m*,*p*-(aminoethyl-aminomethyl) phenethyltrimethoxysilane, were coated onto glass slides and silicon wafers and characterized using contact angle goniometry, ellipsometry, and X-ray photoelectron spectroscopy. Evaluation of the aminosilane-modified surfaces using contact angle measurements, UV−vis spectroscopy, and covalent attachment of a Cy5-conjugated *N*-hydroxysuccinimide ester reporter molecule suggested that derivatization of the surface with APS + PDC resulted in the best overall coverage. Microarrays printed using APS + PDC chemistry to immobilize both amine- and thiol-terminated oligonucleotides resulted in rapid attachment, uniform spot morphology, and minimal background fluorescence. Both amine- and thiol-terminated oligonucleotides showed comparable attachment, although greater attachment and hybridization efficiencies were observed with amine-functionalized molecules at saturating printing densities. The data highlight the influence of surface chemistry on both immobilization and hybridization and, by extrapolation, on microarray data analysis.

Introduction

The potential for microarray-based technologies to conduct rapid, high-throughput, parallel interrogation of thousands of nucleic acid and protein elements has revolutionized biological research and diagnostics. The use of microarray technology has empowered researchers with a systematic tool for applications in DNA and RNA sequence analysis,[1] genotyping of single-nucleotide polymorphisms,[2,3] and the detection of viral and bacterial pathogens.[4,5] The latter application is of particular relevance as recent events involving cases of human inhalation anthrax have highlighted the need for the development of highly specific and sensitive microarray technologies for the detection of food-, air-, and waterborne pathogens. As chemical surface modification and DNA attachment strategies are largely responsible for microarray assay sensitivity, the optimization of these parameters has become a prerequisite for the development of microarray technologies which ideally would be capable of detecting a single pathogenic organism.

A variety of synthetic chemical approaches have been used to immobilize DNA molecules to glass and other surfaces including photoactivatable chemistries,[6−8] hydrogel-based chemistries,[9] poly-lysine-mediated surfaces,[10] dendritic linkers,[11] and homo- or heterobifunctional cross-linkers.[12−16] The driving force in the development of each of these novel attachment methods has been the need to provide (1) a high density of DNA on the surface of the microarray, (2) efficient hybridization reactions for capturing target molecules, (3) low background, (4) stability, (5) reproducibility, and (6) sensitivity. As researchers continue to investigate the contributing variables involved

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in the hybridization between solution phase (targets) and immobilized complementary strands of nucleic acids (probes), fundamental questions that pertain to surface chemistries and their effects on the interactions between target and probe molecules (and thus microarray analysis) have become increasingly germane.[1,17−20] In this study, we examine the surface properties and chemical coupling efficiency of three common aminosilane-functionalized substrates and use DNA sequences unique to the pathogen *Bacillus anthracis* to describe a flexible chemistry that can immobilize both amine- and thiol-terminated oligonucleotides in a microarray format. These results also have implications for the effects of probe surface density on hybridization and assay sensitivity.

Experimental Section

Chemicals. All solvents were of reagent grade quality or higher and were purchased from Sigma-Aldrich and Fisher Scientific, Inc. Ultrapure water (18 MΩ/cm) was obtained from a Milli-Q purification system (Millipore Corp., Bedford, MA). 3-Aminopropyltriethoxysilane (APS), *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (EDA), and *m*,*p*-(aminoethyl-aminomethyl) phenethyltrimethoxysilane (PEDA) were obtained from Gelest, Inc. (Morrisville, PA). 1,4-Phenylene diisothiocyanate (PDC) was purchased from Sigma-Aldrich-Fluka Chemical Corp. (Milwaukee, WI). Oligonucleotides were purchased from Sigma-Genosys (The Woodlands, TX). Glass slides (2.5 cm × 7.5 cm) were purchased from A. Daigger & Co. (Vernon Hills, IL). Monofunctional cyanine 5 (Cy5) fluorescent dyes were purchased from Amersham Biosciences (Piscataway, NJ). Fused silica slides (2.5 cm × 2.5 cm) were obtained from Dell Optics (Fairview, NJ).

Deposition of Aminosilane on Glass Substrates. Acid cleaning and silanization of the glass slides were performed following protocols described previously.[14] Each silane solution was prepared to a final concentration of 2%. Three different deposition solvents (e.g., acidic-methanol, toluene, and an acetic acid solution) were tested with each aminosilane to identify the combination resulting in maximum surface coverage. Contact angle and XPS measurements of each silanized surface were obtained to evaluate surface modification.

Contact Angle Measurements. Static contact angle measurements were obtained with a Rame-Hart goniometer (model no. 100-00-115). Approximately 3 μL of Ultrapure H_2O was deposited onto the modified substrate using a microsyringe. Measurements were recorded immediately postdeposition.

X-ray Photoelectron Spectroscopy (XPS)/Ellipsometry Measurements of Aminosilylated Surfaces. XPS measurements were performed on a Fisons 220i XL X-ray photoelectron spectrometer utilizing a Mg Kα X-ray source (1253.6 eV). The photoelectron takeoff angle was normal to the film surface. The glass substrates were irradiated with a low-voltage electron flood gun during acquisition to compensate for charging. For each sample, a survey scan (0−1100 eV at 50 eV band-pass) and high-resolution scans (20 eV band-pass) over the regions of the specific elements of interest (N, Si, C, and O) were measured. Elemental ratios were derived from the high-resolution peak areas determined after subtraction of a Shirley type inelastic background. Optical ellipsometry was performed with a VASE M-44 multi-wavelength ellipsometer (J. A. Woollan Co., Inc.), to assess film thickness and uniformity for the selected APS-modified silane. Optical constants were determined from acid-cleaned silicon wafers and applied to measurements performed on APS-modified surfaces. The resolution of the ellipsometer was measured at 2 Å.

Reactivity Measurements of the Aminosilylated Substrate. Dilutions of the Cy5-NHS ester (1.4−46 μM) were prepared in sodium borate buffer (100 mM, pH 8.5) and deposited (600 pL/spot) onto APS-, EDA-, and PEDA-treated surfaces with the Packard Biochip I Microarrayer (Packard Biosciences, Meridian, CT). After a 1 h incubation at room temperature (RT), each surface was rinsed with sodium borate buffer and imaged using the ScanArray Lite (GSI Lumonics, Billerica, MA) confocal laser scanning system. Fluorescence intensity measurements were recorded and used to determine which aminosilane would be selected for future experiments.

Homobifunctional Cross-Linking of the Aminosilylated Substrate. The selected APS-modified surface was treated with a 0.2% solution of PDC following a similar protocol established in the literature.[3] PDC-modified surfaces were rinsed twice with methanol and once with acetone and stored desiccated at 4 °C until covalent attachment of the oligonucleotides.

UV−Vis Spectroscopy Analysis of the PDC-Modified Substrate. Fused silica substrates modified with APS and PDC following the above protocol were analyzed spectrophotometrically with a Varian Cary 2400 UV−vis spectrophotometer (Varian Analytical Instruments, Walnut, CA). Acid-cleaned fused silica substrates were used as a reference for UV−vis measurements of APS-modified and APS + PDC modified surfaces. UV−vis scans were performed in a range of absorbance from 190 to 360 nm to determine the successful modification of the surface.

Printing of Amine- and Thiol-Terminated Oligonucleotides. Highly specific oligonucleotide probes were designed, targeting the *cya* and *lef* genes which encode two of the three components of anthrax toxin. Amine- and thiol-terminated *B. anthracis* oligonucleotide probes (*cya* 5′-TCAGGTTTAGTAC-CAGAACATGCAG-3′ and *lef* 5′-GCTTTTGCATATTATATC-GAGCCAC-3′; 25mers) were prepared in sodium borate buffer (100 mM, pH 8.5) and deposited in 20 replicates onto APS + PDC modified glass slides using the Packard Biochip I Microarrayer. Each printed element had a spot diameter of 180 μm, a print volume of 600 pL, and an interelement distance of 300 μm. In addition, amine- and thiol-terminated Cy3-labeled ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) 25mer oligonucleotide probes of known concentrations [0.5 ng/μL to 1.0 μg/μL] were also printed onto APS + PDC modified glass substrates and processed in fashion similar to that of the nonlabeled probes. The fluorescence intensities from the printed Cy3-labeled oligonucleotides of known concentrations were used to generate a standard curve. The surface densities of the immobilized amine- and thiol-terminated oligonucleotides were calculated from the standard curve obtained from Cy3-labeled amine- and thiol-terminated Rubisco oligonucleotides printed in parallel. Once printed, all slides were stored desiccated at 4 °C.

Hybridization to Printed Oligonucleotide Substrates. Oligonucleotide-modified slides were blocked with a 3% bovine serum albumin/casein solution (pH 7.4) for 1 h, rinsed with phosphate buffer saline (pH 7.4), and dried with a N_2 gas stream prior to hybridization. The immobilized probes were treated with a Cy3-labeled complementary target oligonucleotide (4 ng, 200 μL) for 5 min at 50 °C in hybridization buffer (4X SSC, 0.2% SDS). Hybridized slides were then subjected to a series of rinses: (a) hybridization buffer for 3 min at 50 °C, (b) hybridization buffer for 3 min at RT, (c) 1X SSC (twice) for 3 min at RT, and (d) distilled H_2O, followed by air-drying. Slides were subsequently scanned with a GSI Lumonics ScanArray Lite confocal laser scanning system, and the fluorescence intensities were measured with the QuantArray (GSI Lumonics) quantitative analysis software package.

Results

Characterization of Aminosilane-Modified Substrates. Experiments were conducted to determine which aminosilane (APS, EDA, or PEDA) provided optimum monolayer coverage on the glass surface for cross-linking of oligonucleotides and subsequent DNA hybridization. The three aminosilanes were deposited onto acid-cleaned glass surfaces using acidic-methanol, toluene, or acetic acid/H_2O solvents. Contact angle measurements enabled the assessment of surface coverage for each aminosilane/solvent mixture. Previous studies suggested contact angle measurements for APS-modified surfaces were within a

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Table 1. Contact Angles of Aminosilane-Treated Substrates Deposited with Various Solvents and PDC Modification

treatment[a]	acidic-methanol (deg)	toluene (deg)	acetic acid/H_2O (deg)	PDC treatment[b] (deg)
APS-treated	12 ± 1	38 ± 2	17 ± 1	31 ± 1
EDA-treated	20 ± 1	7 ± 4	28 ± 2	28 ± 4
PEDA-treated	43 ± 2	18 ± 2	38 ± 5	31 ± 2

[a] Contact angle measurements were performed in triplicate. Aminosilylation of the substrate was conducted with the respective solvent at 2% silane concentration. [b] PDC treatment of aminosilane slides (acidic-methanol) was performed in a solution of DMF/pyridine (9:1).

12−18° range.[14] As shown in Table 1, contact angle measurements were (12 ± 1°) for APS + acidic-methanol and ellipsometry measurements for the APS-modified surface were 5 ± 1° Å (data not shown). The minimal variability (standard deviations presented in Table 1) using the acidic-methanol solvent solution provided the most consistent surface coverage among the silane/solvent mixtures examined.

These results combined with previously published measurements[14,16,21−23] indicated that silanization of the glass surface with APS in acidic-methanol provided the most uniform chemically modified surface for further experimentation. In addition, XPS measurements of the APS-, EDA-, and PEDA-modified substrates (deposited in acidic-methanol) confirmed an equivalent abundance of nitrogen represented by primary and secondary amines (Figure 2).

Further characterization of the aminosilane-modified substrates (deposited in acidic-methanol) was evaluated based on the reactivity of each aminosilane with a fluorescent reporter molecule (monofunctional Cy5-NHS ester). This was used to determine which aminosilylated surface provided maximum reactivity and availability of primary amines. Depicted in Figure 3A−C are fluorescence images of APS-, EDA-, and PEDA-coated glass surfaces printed with Cy5-NHS ester dye (concentrations from 1.4 to 46.0 μM). Mean fluorescence intensity values for each concentration of Cy5 reporter molecule clearly demonstrated a 3- to 5-fold increase in the fluorescence signal and a greater dynamic signal range on the APS- compared to EDA- and PEDA-modified surfaces (Figure 3D).

PDC Modification of the Aminosilylated Surface. APS-coated slides were reacted with the homobifunctional cross-linker, PDC, to form a stable amide bond and free isothiocyanate moiety for covalent attachment of oligonucleotides (Figure 1B). UV−vis analysis of the APS + PDC treated surface showed an increase in UV absorbance from 190 to 320 nm indicating the presence of the phenyl ring component and conjugation of the PDC cross-linker (Figure 4A). In contrast, UV−vis analysis of the APS-treated surface exhibited minimal to no UV absorbance from wavelengths between 190 and 360 nm (Figure 4B). In addition, contact angle measurements of the APS substrates (12 ± 1°) and APS substrates modified with PDC (31 ± 1°) (Table 1) showed an increase of 19° as a result of the covalent attachment of the PDC cross-linker. This increase in contact angle indicated an enhanced hydrophobicity of the surface that is consistent with successful chemical PDC modification.

Hybridization of Immobilized Amine- and Thiol-Terminated Oligonucleotides. Amine- and thiol-terminated *B. anthracis* oligonucleotides were covalently attached to APS + PDC modified glass slides at spotting densities ranging from 0.3 to 205 fmol/mm^2 (calculated from the standard curves, Figure 5A,B). Hybridization results and fluorescence intensities from the microarrays exhibited in Figure 6A−D showed dramatic differences between the amine- and thiol-terminated probes based on surface probe density. As depicted in Figure 6E, the fluorescence signal intensities for both amine-terminated *cya* and *lef* oligonucleotides reached saturated levels and plateaued at a density of 6.5 and 13.0 fmol/mm^2, respectively. In comparison, fluorescence signal intensities for both thiol-terminated *cya* and *lef* oligonucleotides peaked at density levels of 2.3 and 4.5 fmol/mm^2, respectively (Figure 6F). Unlike the fluorescence intensities obtained for the hybridized amine-terminated probes, hybridized thiol-terminated probes did not reach saturating intensities and showed a decrease in signal intensity at >9.0 fmol/mm^2.

Discussion

In this paper, we investigated a versatile chemistry that utilized the homobifunctional cross-linker PDC to covalently attach both amine- and thiol-terminated oligonucleotides in a microarray format. In utilizing the PDC cross-linking chemistry, we demonstrated the successful attachment of DNA probes and compared the hybridization efficiencies of complementary targets at varying surface probe densities. In addition to extending studies on the immobilization of amine-terminated oligonucleotides,[3] we also provided the first demonstration of this flexible surface chemistry to covalently couple thiol-terminated oligonucleotides.

A number of chemical modification strategies have been employed by researchers for the attachment of oligonucleotides to glass surfaces. Within each one of these strategies exist a number of advantages and limitations. Poly-lysine coatings and noncovalent attachment (passive) methods using hydrophobic interactions between DNA and the surface have been used but often are susceptible to detachment of the DNA films from the microarray surface when exposed to stringent hybridization conditions (high salt and/or high temperatures). These substrates have resulted in experimental inconsistencies that beget inconclusive data interpretation. The design and microfabrication of DNA microarrays using in situ photolithographic synthetic chemical procedures have become an increasingly popular alternative. This state-of-the-art methodology provides the capability of producing and interrogating hundreds of thousands of microarrayed elements per assay.[7,8] Although this technique alleviates the deficiencies inherent in noncovalent attachment strategies, issues related to cost and the length of the capture probe are critical limitations of this approach. Covalent coupling chemistries provide the flexibility to combine the beneficial attributes of noncovalent attachment methodologies (ligand selection, length, and cost) and in situ photolithographic attachment methodologies (stable and reproducible covalent chemistry).

Zammatteo et al.[1] and Chrisey et al.[14,15] have stressed the need for efficient covalent coupling chemistries (e.g., carbodiimide-mediated coupling, aldehyde activation, heterobifunctional cross-linkers) that can provide stability and consistency to DNA microarrays. Guo et al.[3] and Lindroos et al.[2] cited the use of isothiocyanate-modified

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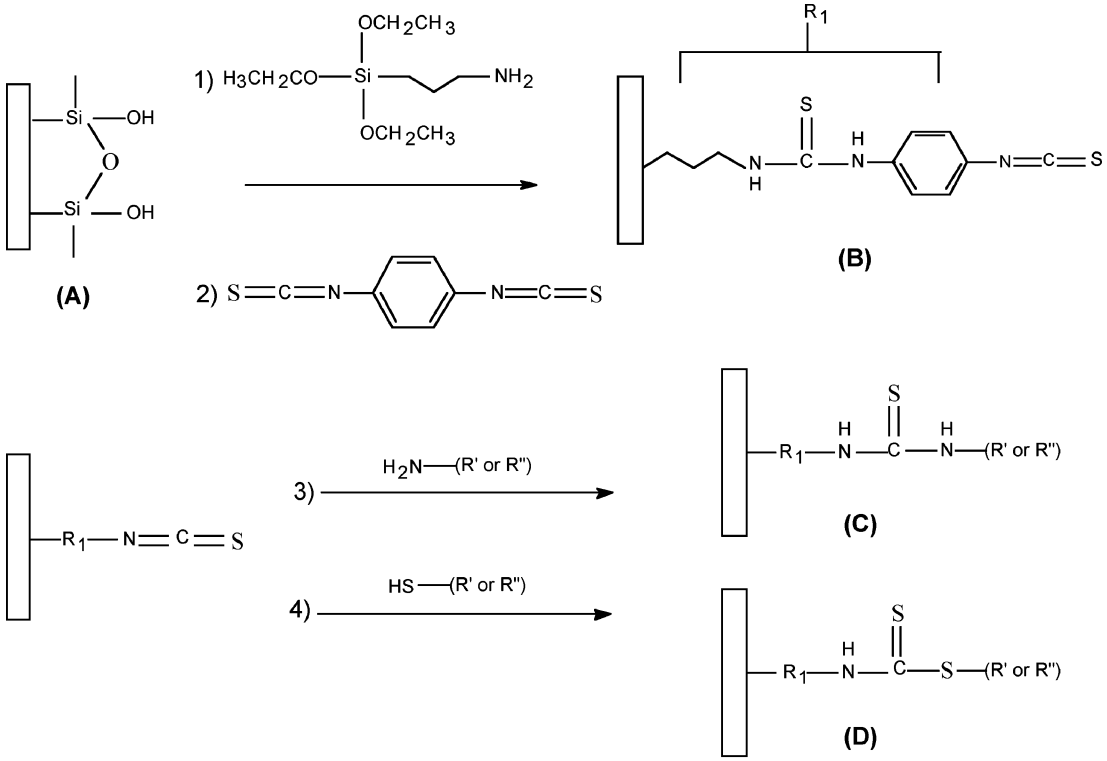

Figure 1. Structural schematic depicting reaction mechanisms for the covalent attachment of amine- and thiol-terminated oligonucleotides to a glass substrate: (A) initial formation of surface hydroxyl groups on the glass substrate by acid cleaning for (B) covalent attachment of APS and PDC; cross-linking of (C) amine- and (D) thiol-terminated oligonucleotides to the PDC-modified surface through amide bond and dithiocarbamate bond linkage.

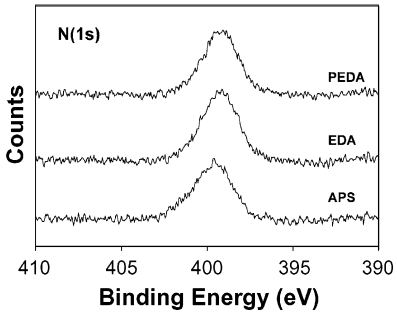

Figure 2. Comparison of high-resolution N(1s) XPS spectra for APS, EDA, and PEDA films. The N/Si ratios derived from the films were 0.106, 0.099, and 0.109, respectively.

substrates for the analysis of genetic polymorphisms on oligonucleotide arrays and demonstrated that covalent attachment of amine-terminated oligonucleotides through an isothiocyanate-based chemistry provided the best signal-to-noise ratio. These results, combined with the facts that (1) PDC is relatively inexpensive compared to the cost of heterobifunctional cross-linkers, (2) this chemistry provides long-term stability, (3) PDC promotes the efficient cross-linking of amine-terminated oligonucleotides, (4) this platform is versatile and novel for coupling thiol-terminated oligonucleotides, and (5) this attachment chemistry has potential application for covalent attachment of non-nucleic acid molecules including small organic compounds, antibodies, and peptides, provided the framework for further investigating the isothiocyanate-based chemistry.

In our efforts to characterize the APS + PDC modified surface, a number of surface characterization techniques

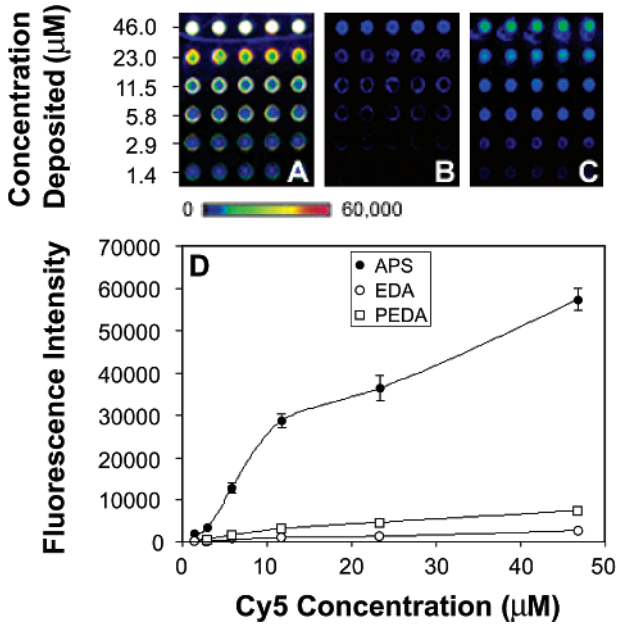

Figure 3. Fluorescence microarray image depicting reactivity of a Cy5 *N*-hydroxysuccinimide ester reporter molecule on aminosilane-derivatized glass substrates: (A) APS, (B) EDA, and (C) PEDA. Five representative replicates of 20 are shown. (D) Plotted representation of panels A−C showing differences in fluorescence intensity. The data shown represent means ± standard deviation (SD) of 20 replicates.

(e.g., contact angle, ellipsometry, XPS, and UV−vis spectroscopy) were utilized. These techniques were used to measure monolayer coverage of the selected APS-modified substrate and enabled identification of the most consistent

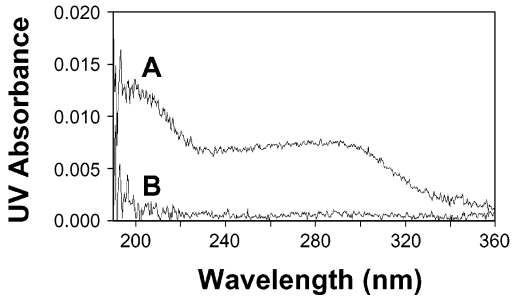

Figure 4. UV−vis spectroscopy measurements of (A) APS + PDC modified and (B) APS-modified surfaces on a fused silica surface. The UV−vis wavelength scan was performed from 190 to 360 nm.

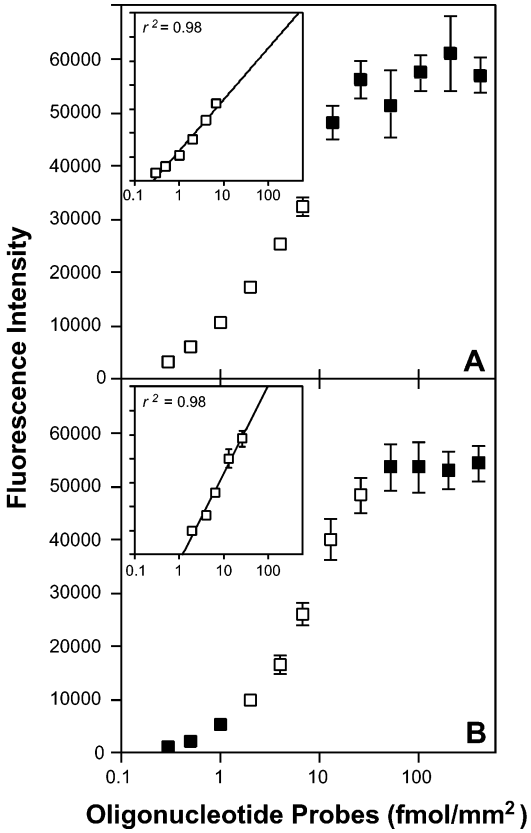

Figure 5. Fluorescence intensity response from (A) amine- and (B) thiol-terminated Cy3-labeled Rubisco oligonucleotides printed onto APS + PDC modified glass slides. Oligonucleotides were deposited at concentrations from 0.2 to 430 fmol/mm^2. The white symbols are isolated in the graph insets and represent the respective linear range of each standard curve. The data shown represent means ± SD of 10 replicates. Both coefficient of determination (r^2) values were equal to 0.98.

surface coverage that was similar to results in previously published literature.[14,16,21−23] XPS measurements of the APS-, EDA-, and PEDA-modified substrates also confirmed an equivalent abundance of nitrogen represented by primary and secondary amines (Figure 2). Further characterization of the aminosilane-modified substrates was based on the reactivity of each aminosilane with a monofunctional Cy5-NHS ester. The 3- to 5-fold increase in fluorescence signal demonstrated a greater dynamic signal range on the APS- compared to EDA- and PEDA-modified surfaces. The reduced fluorescence signal intensities for EDA- and PEDA-modified surfaces, in comparison to APS, suggest that both amines from EDA and PEDA are unlikely to be available for chemical reactivity due to steric constraints. Thus, of the three

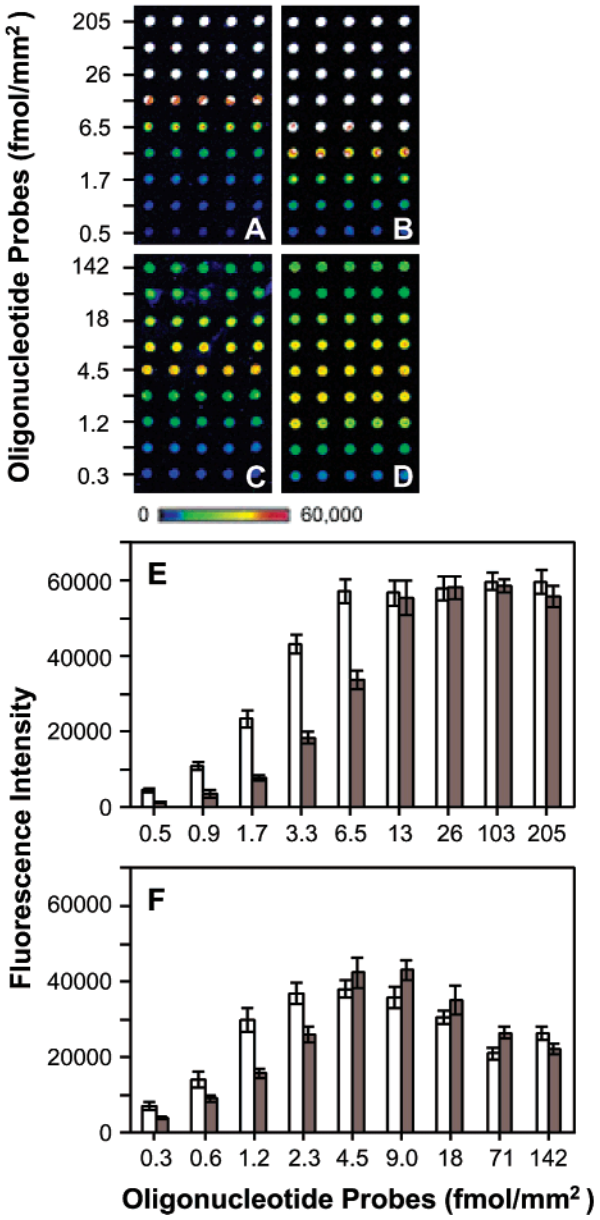

Figure 6. Fluorescence microarray images of immobilized (A) amine-terminated *lef* oligonucleotides, (B) amine-terminated *cya* oligonucleotides, (C) thiol-terminated *lef* oligonucleotides, and (D) thiol-terminated *cya* oligonucleotides. Each immobilized probe was hybridized with a complementary *lef* or *cya* Cy3-labeled target oligonucleotide. Five representative replicates of 20 are shown. Graphical representation of fluorescence intensity measurements of hybridized Cy3-labeled complementary oligonucleotide targets to (E) amine-terminated probes (A,B) and (F) thiol-terminated probes (C,D). Gray bars = *lef* DNA probes, white bars = *cya* DNA probes. The data shown represent means ± SD of 20 replicates.

aminosilanes examined, APS shows the best overall surface coverage and reactivity.

Characterization of the APS + PDC modified surface by UV−vis analysis showed a significant increase in UV absorbance from 190 to 320 nm. In addition, contact angle measurements of the APS + PDC modified surface demonstrated an increase of 19°. This increase in absorbance and contact angle indicated the presence of the phenyl ring component and conjugation of the PDC cross-linker. The increase in contact angle from the PDC-cross-linked substrate also suggested an enhanced hydrophobicity of the surface that was consistent with successful chemical PDC modification.

As DNA microarrays evolve and novel immobilization strategies are developed, a continued effort by researchers must focus on understanding the fundamental molecular interaction of DNA on surfaces and the effect surface probe density has on the hybridization of molecular targets. As reported by Peterson et al.,[26] oligonucleotide probe density has been identified as a controlling factor for the efficiency of target capture as well as the kinetics of the target/probe hybridization. Some reported surface densities of oligonucleotides varied with each application from 0.1 pmol/mm^2 on aminated polypropylene to 150 fmol/mm^2 using dendrimer-activated solid supports.[11,17] Capture probe concentrations have also been reported at 300 fmol/mm^2 and ranged from 2.0×10^{12} to 12.0×10^{12} molecules/cm^2 in hybridization experiments conducted by surface plasmon resonance (SPR) spectroscopy.[1,26] Spacer length or tethering of the oligonucleotide also has a marked effect on hybridization.[18] In an effort to address the surface probe density and its related effect on hybridization using the PDC chemistry, a series of hybridization experiments were conducted with varying densities of immobilized amine- and thiol-terminated *B. anthracis* oligonucleotides.

Under our experimental conditions, hybridization results yielded saturated levels of fluorescence with the immobilized amine-terminated oligonucleotides. The most significant observation, however, was the decrease in fluorescence intensity observed with thiol-terminated oligonucleotides at densities of $\geq$9.0 fmol/mm^2 (Figure 6B). This decrease in hybridization may be attributed to the reactivity of highly concentrated thiol moieties in solution. The data also suggest that the highly electrophilic central carbon of the PDC cross-linker may have undergone successive nucleophilic addition with vicinal thiol-terminated oligonucleotides creating a densely packed localized environment with repulsive electrostatic and steric interactions that could inhibit hybridization.[24−26] Although we anticipated saturated fluorescent signals with an increasing density of the immobilized DNA (as seen in Figure 6A,B,E), the decrease in hybridization observed at higher densities of printed thiol-terminated oligonucleotides was unexpected (Figure 6C,D,F). One possible interpretation of the observed signal decrease in the thiol-terminated probe/target hybridization experiment was that fluorescence quenching occurred between densely packed fluorescence probes as documented by McGall et al.[27] However, no such evidence of fluorescence quenching was observed in the present study. Experimental data from fluorescence microarrays with direct immobilization of Cy3-labeled amine- and thiol-terminated oligonucleotide probes onto the PDC-modified surface at high surface probe densities (up to 430 fmol/mm^2) exhibited no signs of fluorescence quenching (Figure 5A,B). These data also highlight the importance of the selection of fluorescence probes that do not photobleach and are water soluble, unlike fluorescein dyes. The data presented demonstrate the influence of chemistry-dependent effects on immobilization and hybridization at high probe densities, which ultimately impact the reliability and sensitivity of microarray analysis.

Conclusion

An experimental study was conducted comparing the hybridization efficiency of immobilized DNA oligonucleotides on glass substrates treated with APS and cross-linked with the homobifunctional cross-linker, PDC. Amine- and thiol-terminated *B. anthracis* probes were covalently attached to the PDC cross-linker at varied densities and hybridized with Cy3-labeled complementary targets. Hybridization experiments showed significant differences between microarrays spotted with amine- and thiol-terminated oligonucleotides. Fluorescence signals for immobilized *cya* and *lef* amine-terminated probes plateaued at densities of $\geq$6.5 and 13.0 fmol/mm^2, respectively. In contrast, target hybridization to the immobilized thiol-terminated probes resulted in a density-dependent decrease at $\geq$9.0 fmol/mm^2. Thus, although the use of a PDC cross-linker provides for a flexible and cost-effective surface chemistry for covalent attachment of both amine- and thiol-terminated oligonucleotides, immobilization of amine-terminated DNA for the purpose of hybridization was significantly more efficient. The comparative success of the APS + PDC chemistry observed also provides a viable alternative to other attachment strategies used to detect a variety of molecular interactions on microarrays. As a result, a reproducible and stable substrate was achieved with little to no background interference and high fluorescence signal responses that can serve as a template for the subsequent development and design of microarray-based research and diagnostic technologies.

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