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Application of physicochemically modified silicon substrates as reverse phase protein microarrays

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Abstract

Physicochemically modified silicon substrates can provide a high quality alternative to nitrocellulose-coated glass slides for use in reverse-phase protein microarrays. Enhancement of protein microarray sensitivities is an important goal, especially because molecular targets within patient tissues exist in low abundance. The ideal array substrate has a high protein binding affinity and low intrinsic background signal. Silicon, which has low intrinsic autofluorescence, is being explored as a potential microarray surface. In a previous paper (Nijdam, A.J., Cheng, M.M.C., Fedele, R., Geho, D.H., Herrmann, P., Killian, K., Espina, V., Petricoin, E.F., Liotta, L.A., Ferrari, M. (2007) Physicochemically Modified Silicon As Substrate For Protein Microarrays *Biomater*, 28, 550-558), it is shown that physicochemical modification of silicon substrates increases the binding of protein to silicon to a level comparable with that of nitrocellulose. Here, we apply such substrates in a reverse-phase protein microarray setting in two model systems.

Introduction

Reverse phase protein microarrays enable disease-related proteins derived from tumor cells within patient biopsies to be profiled in a high throughput manner (1-6). With reverse phase protein microarrays, a heterogeneous mixture of proteins from a cellular lysate are non-covalently immobilized onto a substrate surface using an arrayer similar to those used in DNA

microarray fabrication. By definition, a reverse phase protein microarray has the protein of interest immobilized on a surface (5,8).

Cellular lysates may be derived from cells grown in culture or from discrete cell populations purified from tissue specimens using laser capture microdissection (9-14). The protein lysate spots can then be probed using antibodies specific to the protein isoforms of interest for a given disease (Fig. 1a). Some of the most valuable protein analytes are in low abundance because they exist in low copy numbers in cells, or the tissue sample is derived from a small core biopsy.

Reverse phase protein microarrays are especially useful for measuring phospho-specific events since these epitopes are usually masked and require denaturation of the target molecule prior to analysis as was done in this paper. The presence of a bound primary antibody can then be visualized through the use of several technologies. For example, the presence of a bound biotinylated antibody can be detected by streptavidin linked to a reporter molecule (Fig. 1b). This allows a single reporter system to be applied to various, different primary probes. In particular, post-translationally modified isoforms of proteins, such as phosphorylated proteins, can be studied in this manner; a functional endpoint that has eluded gene microarrays. Another advantage is reporting on a functional endpoint which has eluded gene microarrays. This approach has yielded insight into numerous types of cancer (12,13,15-20).

Application of emerging nanotechnologies to biology and medicine has produced fluorescently labeled probes through the conjugation of inorganic fluorophores, quantum dots to biological interacting molecules (21-24). Quantum dots are fluorescent, semiconducting nanocrystals, ranging in size from 1 to 10 nm. These nanoparticle labeling agents possess broadband excitation bandwidths with narrow emission bandwidths, large extinction coefficients, and very high quantum yields that result in bright fluorescence. One limitation of protein-based molecular profiling is the lack of intrinsic amplification that PCR-based technologies provide for DNA-based studies. Beyond engineering efforts that alter enzymatic amplification chemistries for label detection, one way to boost the signal in protein microarrays is to use an array substrate with high protein binding potential.

Currently, the substrate for reverse phase protein microarrays commonly consists of nitrocellulose coated onto a glass surface (25). In common use, reverse phase protein microarrays using chromogenic reporter technologies are limited to the detection of one protein target per protein spot. The advent of non-organic fluorophores, promises a more versatile, multiplexed reporter system (5,26).

Here, we focus on physicochemically modified silicon as an alternative for nitrocellulose coated glass substrates. While nitrocellulose has excellent protein binding properties, its autofluorescence presents a significant background fluorescent signal (Fig. 2, left) (27). Silicon is an attractive potential substrate because it has low intrinsic autofluorescence when excited by wavelengths used in fluorescent protein microarrays. (Fig. 2, right). As an alternative to nitrocellulose coated glass substrates, chemically modified glass substrates which are used in DNA and RNA microarray techniques have also been investigated (28-30). We have not considered such glass-based substrates here. First, the surface area of chemically modified glass substrates is not adequate, leading to low sensitivity. The surface area of glass substrates is up to ten thousand times lower than that of nitrocellulose coated glass slides, depending on the thickness of the nitrocellulose. Second, while the surface area of silicon can easily be modified by readily available clean-room technology, this is not possible with glass.

In order to directly compare the protein binding potentials of nitrocellulose and silicon, we devised an enzyme-based assay to objectively measure the level of protein binding to each (27). Using this system, multiple substrates with binding potentials comparable to nitrocellulose-backed glass slides were generated using reactive ion etching and/or chemical

modifications of silicon. The aim of that work was to evaluate what physicochemical modifications maximize protein adherence to the silicon substrates. The system facilitated a head-to-head evaluation of binding potentials possessed by tailored silicon substrates. Pure biotinylated protein was used as the analyte. The reactive ion etching increased the general roughness of the silicon substrates. Further chemical treatment with mercaptopropyltrimethoxysilane (MPTMS) and 3-aminopropyltriethoxysilane (APTES) increased the protein affinity of the silicon substrates. Chemical modification with poly (ethylene glycol) (PEG) and PEG-N-hydroxysuccinimide was not successful (27).

To test the versatility of physicochemically modified silicon substrates as an alternative for nitrocellulose-coated glass substrates, this current study tested physicochemically modified silicon substrates in two model systems. The first model system was biotinylated albumin using quantum dots. The second model was (phosphorylated) epidermal growth factor (EGF) in unpurified A431 cell lysates, in a reverse-phase protein microarray setting, in which pegylated streptavidin quantum dots were used to detect ERK signaling proteins (26).

The biotinylated albumin model with quantum dots was chosen to demonstrate the versatility of physicochemically modified silicon substrates by showing that the fluorescence from the quantum dots may be invisible on nitrocellulose-coated glass slides, while it was visible on physicochemically modified silicon substrates. The reverse-phase protein microarray model was chosen to show the versatility of the physicochemically modified silicon substrates.

Experimental

Silicon microarray fabrication

Silicon roughening: reactive ion etching—Silicon microarray fabrication was performed largely along the lines that we described previously (27). In short, 4" silicon wafers (P-doped, 1-20 Ω -cm (Silicon Quest International Inc., Santa Clara, CA, USA)) were cleaned in a Semitool SAT (Spray Acid Tool) according to a protocol including one ozone step, one fluorozone step, one diluted HF step (all @ 95 °C), with several rinsing steps. Wafers were reactive ion etched (RIE) in a Lam AutoEtch 490, rebuilt by Aspect Systems Inc @ 250 mTorr, 1.4 cm electrode gap, and 50 sccm (standard cubic centimeters) each of helium, oxygen and hexafluorosulfide. RIE is a form of plasma assisted etching in which the substrate is bombarded with (positive) ions from the plasma by an applied bias (31). The wafers were etched for 20 min or 180 min. The wafers were then cleaned again in the Semitool SAT and diced into 25 $\times$ 75 mm samples.

Chemical modification of roughened substrates—3-aminopropyltriethoxysilane (APTES), dry toluene, mercaptopropyltrimethoxysilane (MPTMS), and 2-propanol (IPA) were obtained from Fisher Scientific, Columbus, OH, USA. In the following, all chemicals were obtained from Fisher, unless otherwise indicated. The treatments were found in numerous places in the literature, as such, a representative citation is provided. These were adapted slightly to the requirements of this study.

APTES treatment: APTES was dissolved 10 % by volume in dry toluene. The previously roughened silicon wafers were immersed with mild stirring overnight at room temperature, removed from the solution, rinsed with toluene and dried in vacuo (32).

MPTMS treatment: MPTMS was dissolved 2 % by volume in IPA. The previously roughened silicon wafers were immersed with mild stirring overnight at room temperature, removed from the solution, rinsed with IPA and dried in vacuo (33).

Blank silicon is covered with a thin native oxide layer which is 10 to 15 nm thick, which in aqueous solutions becomes hydrolyzed leading to a surface terminated in silanol group. APTES treatment resulted in a silicon surface that is functionalized with amino (-NH₂) groups. MPTMS treatment lead to the deposition of thiol (-SH) groups onto the surface of silicon.

Sample spotting and detection—The samples were spotted onto the physicochemically modified silicon or nitrocellulose-coated glass (FASTslides, Whatman Inc, Florham Park, NJ, USA) substrates with the robotic 428 Arrayer (Affymetrix Inc., Santa Clara, CA, USA). The instrument utilized a pin and ring technology to array 8 spots simultaneously. Each pin had a diameter of 125 µm, and there was one strike per spot. The pins and rings were washed twice in flowing water for 8 seconds each time followed by 8 seconds of vacuum to dry the pins and rings. A pin and ring calibration was performed before each run, followed by a wash in 10 % (v/v) Clorox (Kroger Co., Columbus, OH, USA) and isopropanol, for 10 min. each, following the calibration and before the spotting.

The slides were scanned with a 428 Scanner which is a confocal laser microscope based system with a 10 µm resolution. The gain for the detector was set at various values to maximize signal without saturation, e.g. 55 to 70dB. The maximum gain or setting for sensitivity was 70. The slides were scanned with the Cy5 dye setting which utilizes a laser with a 635nm emission and a filter that detects fluorescence emission at 655 ± 12nm.

For each slide the 428 scanner generated a images which were processed by manually identifying each spot on the slide, calculating the average pixel intensity value for the region inside the spot and the average pixel value for the region just above the spot as a control. The average spot and control values for each spot in the image were written to an Excel file for statistical analysis.

Biotinylated albumin reverse-phase protein microarrays

Selected slides were spotted with dilutions of biotinylated bovine serum albumin (Fisher Scientific, Columbus, OH, USA) in PBS (phosphate buffered saline) ranging from 100 µg/mL to 1 pg/mL. After spotting, the slides were immersed in an I-block solution (50 mL of PBS, 0.1 I-block powder (Applied Biosystems, Foster City, CA, USA), heated until dissolved, then cooled, with 50 µL of Tween-20) overnight @ 4°C. Slides were immersed in Dako blocking solution (DakoCytomation, Carpinteria, CA, USA) for 15 min and washed with Dako wash buffer and PBS, three times each for 2 min and spin dried. Subsequently, 200 µL of 1:100 diluted PEG-SAV-Qdots (655 nm, Invitrogen, CA, USA) was sprinkled on the slides under a cover slide for 15 min, and slides were washed twice for 2 min in PBS and spin dried.

A431 Reverse-phase protein microarrays

A431 cell growth and lysing—A431 human epidermoid carcinoma cells (American Type Culture Collection, Manassas, VA, USA) were grown in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA, USA), 10% heat-inactivated FBS (Invitrogen), 1% nystatin (Sigma, St. Louis, MO, USA), 4 mM L-glutamine (Invitrogen), and 50 µg/ml gentamycin (Invitrogen). Cells were maintained at 37 °C with 5% CO₂. For protein isolation, confluent cultures were changed to serum-free media for 24 hrs, at which point cells were treated with 100 ng/mL epidermal growth factor (EGF, Invitrogen) for 5 min. Cells were pelleted by centrifugation and washed with PBS before protein isolation.

Cells were lysed by adding 1.5mL of boiling lysis buffer (1% SDS, 1.0mM Na o-vanadate, 10mM Tris pH 7.4). The mixture was boiled for another 5 min and passed several times through a 26 gauge needle to reduce the viscosity. Insoluble pellets were discarded after centrifugation.

The amount of protein was quantified using a BCA protein concentration assay (Pierce, Rockford, IL, USA) and diluted to 1 mg/mL.

Reverse-phase protein microarrays—A431 cell lysates were spotted in a serial dilution from 1:1 to 1:64 in a T-PER-SDS buffer (1mL of T-PER in 950 μ L of SDS and 50 μ L of β -mercaptoethanol). After spotting, the slides were immersed in 1x Re-Blot solution for 15 min, washed twice in PBS for 5 min and submerged in I-block solution for 2h. The slides were immersed in 30% H_2O_2 and rinsed three times for 2 min in TPS (DakoCytomation). Subsequently, 200 μ L of avidin block, biotin block and protein block (all DakoCytomation) were applied under a cover slip for 10, 10 and 5 min respectively. After each step, the slides were washed three times in TPS for 2 min. The primary (p44/42 MAP Kinase Antibody, Cell Signaling Technology, Inc., Danvers, MA, USA) and secondary antibodies (Biotinylated Anti-Rabbit IgG (H+L), made in goat, Vector Laboratories, Burlingame, CA USA) were applied for 30 and 15 min, respectively, followed by a TPS wash step. Furthermore, 200 μ L of streptavidin-biotin complex solution and 200 μ L of amplification solution were added under a cover slide, each followed by three TPS washes of 2 min. Finally, 200 μ L of 1:100 diluted qdots (Invitrogen, Hayward, CA, USA) were added under a cover slide, followed by two final washes in PBS for 5 min.

Results and discussion

Roughened silicon

The wafers were etched 20 or 180 min to increase the surface area. As shown in previous work (27), reactive ion etching yielded a significant increase in the roughness of the silicon. By visual inspection, the increased roughness was observed by noting an altered visual appearance of the wafer surfaces. The cross sectional scanning electrode views demonstrated the marked differences in the silicon surface found as the etching increased from 20 to 180 minutes. A mountain-like topography, with peaks and valleys, was generated at longer etching times (Fig. 3), whereas unetched silicon is nearly atomically flat.

The performance of physicochemically modified silicon substrates as protein microarray substrates was compared to nitrocellulose-coated glass substrates in two ways. First, it was explored if biotinylated albumin could be detected on the substrates using quantum dots. Second, the total protein lysate of A431 cells, previously treated with epidermal growth factor, was probed for p44/42 MAP Kinase antigens in a reverse-phase protein microarray setting. In each experiment, a dilution curve of the analyte was spotted onto blank untreated silicon, the three variations of the physicochemically modified silicon substrates and on nitrocellulose-coated glass substrates.

In previous work (27), it was shown that 180 min of RIE etching and treatment with APTES or MPTMS yields silicon that binds an equivalent amount of albumin as nitrocellulose. In fact, it was also shown, that for the MPTMS treated silicon substrates, 20 min of RIE etch lead to an optimal amount of albumin bound, even in excess of the amount that bound to the nitrocellulose substrate.

Biotinylated albumin detected by quantum-dots

In order to test the feasibility of physicochemically modified silicon substrates as protein microarrays by using quantum-dots, silicon substrates were first tested in a relatively simple set-up for the detection of biotinylated albumin (fig. 1b). A dilution series of biotinylated albumin was spotted onto the substrates. After two blocking steps, to inhibit non-specific binding, and several washing steps, streptavidin-pegylated quantum dots were allowed to bind to the biotin-groups on the albumin. Since the interaction between streptavidin and biotin is

one of the strongest non-covalent bonds known (34), this model was an excellent model to study the feasibility of physicochemically modified silicon substrates as an alternative for nitrocellulose substrates, using fluorescent detection.

Five different physicochemical modalities were tested and compared to nitrocellulose coated glass: blank unetched silicon; 20 and 180 min RIE etched silicon, treated with MPTMS; and 180 RIE etched silicon, treated with APTES. A dilution curve ranging from 100 µg/mL to 5 pg/mL of biotinylated albumin and PBS as a control was spotted onto the slides. The dilution curve was repeated eight times on each substrate and the experiment was performed on three substrates of each physicochemical modality, leading to a total of twenty-four curves per physicochemical modality.

Fig. 4 shows a comparison of the dilution curves of biotinylated albumin on an untreated, blank silicon substrate, as well as a 20 min RIE etched, MPTMS treated silicon substrate. A marked increase in fluorescence is clear for the physicochemically modified substrate. Where only spots of 5 and 10 µg/mL are visible on the blank silicon substrate, the physicochemically modified substrate shows spots down to the 10 ng/mL-500 pg/mL range. Individual spots appear to be visible for lower values, but these fall within the background noise of the reading (see below). Furthermore, there is a small phantom spotting effect seen on the lower four spots (PBS) of the physicochemically modified slide. It is unclear what causes these spots. Nevertheless, these results confirm that substrates that have been roughened by RIE etching and MPTMS treatment, show increased albumin and therefore protein binding.

Fig. 5 shows the results for spotting on 180 min etched, MPTMS and APTES treated silicon substrates, as well as for the spotting on nitrocellulose. The 180 min RIE etched, MPTMS substrate shows comparable results as the 20 min RIE etched MPTMS substrate. However, the spot morphology of the 180 min RIE etched substrates treated with MPTMS as well as APTES show is less defined. This is attributed to the increased roughness of the surface of the 180 min RIE etched substrate as compared to the 20 min RIE etched surface. Also, the protocols for these experiments are based on protocols that were previously developed for optimal spot morphology on nitrocellulose coated glass substrates (26). Spotting morphology can be altered by the use of surfactants. Obviously, there is a significant difference between the surface properties of physicochemically modified silicon substrates and nitrocellulose-coated glass slides. Optimization of the protocols for physicochemically modified silicon substrates is therefore expected to yield a markedly better spot morphology.

The 180 min RIE etched, APTES treated substrate demonstrates a markedly increased background signal, which is attributed to non-specific binding of the streptavidin conjugated pegylated quantum dots to the amino-groups of APTES. However, statistical analysis of the difference between the spot signal and the background signal does not yield significant difference with the MPTMS treated slides. The nitrocellulose-coated glass slide does show well defined spots, but due to fluorescent background signal of the nitrocellulose, no information can be obtained from these apparent spots; the variation in the background fluorescence is larger than the spot intensities. Furthermore, the PBS spots are not significantly different from the other spots. This makes the distinction between real and phantom spots impossible.

Our physicochemical modification experiments described in (27) which tested the total amount of albumin bound to the physicochemically modified substrates, determined that the 20 min RIE etched, MPTMS treated substrates bound the greatest amount of albumin compared to any other modification. In the current experiments described in this paper, we found that the 180 RIE etched, APTES treated substrates has the highest signal, corrected for the background. Although these results appear to be contradictory, they are not. In our previous experiments,

the surfaces of the modified substrates were entirely covered with albumin, which was detected by a colorimetric assay. In the present studies only a portion of the surface is spotted, and the detection method is based on using fluorescence. The fluorescent signal is influenced by the spot morphology, as well as the background subtraction. Overall, the results of both experiments illustrate the same point: the physicochemically modification silicon substrate leads to enhanced albumin binding.

While the nitrocellulose-coated glass slides yielded inconclusive results, physicochemically modified silicon substrates yielded good results. Fig. 6 shows the averaged intensities (shown as arbitrary intensity units) of the spots for the lower albumin concentrations. It is obvious that the physicochemical modification yields a higher signal than the untreated blank silicon. The results in Fig. 6 clearly demonstrate that the 180 min RIE etched, APTES treated silicon substrate is the substrate with the best signal, even though the background signal is markedly higher than that of the MPTMS treated silicon substrates. For the higher concentrations (not shown), the intensity the signal increase about two orders of magnitude.

Physicochemically modified silicon substrates used as reverse-phase protein microarrays

In order to test the feasibility of the use of the physicochemically modified silicon substrates in a reverse-phase protein microarray, the silicon substrates were tested in a slight modification of a previously well-tested reverse-phase protein microarray setting. The lysates for A431 cells were spotted in a known dilution series ranging from 1:1 to 1:32 onto the substrates, which corresponds with a range from 0.50 mg/mL to 32 μ g/mL. Subsequently, several blocking steps were performed to prevent non-specific binding. Then, the lysates were probed with a primary antibody, p44/42 MAP Kinase Antibody, followed by a secondary antibody, biotinylated Anti-Rabbit IgG (H+L), made in goat. An amplification step was performed so that eventually multiple streptavidin-conjugated quantum dots can bind to the biotin groups on the secondary antibody. Schematically, this process is represented in Fig. 1a (5). The same set of substrates was tested in the same experimental series as described above.

Fig. 7 shows five representative substrates. On each substrate two series of four dilution curves were spotted, as indicated above the substrates. The physicochemical modification increased the amount of protein (this time from the lysates) that was bound to the untreated, unetched silicon substrate. Surprisingly, the APTES treated substrate yielded no spots whatsoever. It is likely however that the spots were invisible due to the high background signal. Also, the spot size was larger than in the biotinylated albumin experiments (see below). It could be argued that with a more concentrated spot size, a signal would have been visible. If the quantum dots still contained unreacted carboxylic acid groups, non-specific binding might occur between those $-COOH$ groups and the amino-groups on the surface. An unknown factor (according to the manufacturer) was whether there are unreacted carboxylic groups that are also sterically available for binding to a solid surface. The 20 min RIE etched, MPTMS treated substrate showed brighter spots than the 180 min etched, MPTMS treated substrate. This is in agreement with our earlier experiments (27) in which it has been determined that more albumin binds to 20 min RIE etched, MPTMS treated substrates than to 180 min RIE etched, MPTMS treated substrates. By visual inspection, the 20 min RIE etched substrates showed spots down to 1:32 dilution in the left example, and down to 1:4 in the right series in Fig. 7. The 180 min RIE etched substrate however, only showed readable spots for the 1:1 and 1:2 spots. The detection limit for the 20 min RIE-etched, MPTMS-treated silicon substrates is consistent with previous reports (26, Fig. 2b and c).

The spot morphology on the silicon substrates was less defined than on the nitrocellulose-coated glass substrate. In fact, the spots of the highest concentration merged into the shape of a line, or a dash. It should be stressed again that protocols were used which were optimized for nitrocellulose-coated glass substrates. Those protocols were not only optimized for the highest

efficiency of the biochemistry involved, but also for more physical-chemical parameters such as the spot morphology, which was determined largely by the use and concentration of surfactants; T-PER and SDS. Obviously, the surface properties of the organic polymer nitrocellulose were quite different from elemental silicon. Furthermore, our cell lysates contained many molecules from the cell membranes that have surfactant properties. The concentration of these molecules changed significantly throughout the dilution series, as it was proportional with the concentration of our lysates. This explained why the spots had a different morphology for the higher concentrations, as compared to the lower concentrations.

Optimization of surface properties is crucial to reaching optimal results, but not trivial. (35). In addition, the structure of the materials is very different. Nitrocellulose is a porous polymer (Fig. 3d in (27)), whereas these silicon substrates are roughened by reactive ion etching (Fig. 3). Lastly, nitrocellulose is a soft material, and the pin strikes that deposit the sample onto the substrate create small indentations in the nitrocellulose layer. The transferred sample drop will be located in the dent. This is partly the cause of the phantom spotting effect in Fig. 5. Obviously, such dents are not fabricated in the silicon substrates, as silicon is much harder than nitrocellulose. With proper optimization, however, we believe that the spot morphology can be significantly improved on the physicochemically modified silicon substrates. Better spot morphology will also increase the repeatability of the experiments.

To illustrate the influence of the solvent on the spot morphology, three short dilution series of biotinylated albumin were dissolved in three different solvents and spotted. The solvents were PBS (same as Fig. 4), T-PER/SDS/ β -mercaptoethanol (same as Fig. 7) and urea/CHAPS (8M urea, 4 % (w/v) CHAPS ((2(3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate), and 40 mM Tris) – a common solvent for cell lysates. The results are shown in Fig. 8. The dilution series of biotinylated albumin in PBS was relatively well defined, compared to two series, where the T-PER series was significantly blurrier, and the dots merge almost completely in the urea series.

Overall though, we showed that physicochemically modified silicon substrates were used in a reverse-phase protein microarray setting for the detection of MAP Kinase proteins in unpurified A431 cell lysates. The results were comparable with those on traditionally used nitrocellulose-coated glass slides, especially for the 20 min RIE etched, MPTMS treated substrates. With proper optimization, this technology could yield enormous advantages in the field of pathology by opening the detection of low molecular weight cancer biomarkers from unpurified samples in lower concentrations than possible with current technology. In addition, this approach builds on technology such as the substrate spotter and scanner is readily available in many (university) laboratories. Thus, this approach can be easily and cheaply implemented.

In conclusion, it was shown that physicochemically modified silicon substrates could be used in a reverse-phase protein microarray setting by two simple models. First, biotinylated albumin was detected by quantum dots that were not detectable on traditionally used nitrocellulose-coated glass substrates. Second, it was shown that detection of (phosphorylated) MAP Kinase in unpurified A431 cell lysates was also possible with a similar detection range as in nitrocellulose-coated glass substrates. With better optimization of the protocols, we believe that the physicochemically modified silicon substrates can have a higher detection than nitrocellulose-coated glass substrates.

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Fig. 1. (a): Schematic representation of a reverse-phase microarray. In the reverse phase protein microarray, proteins from cellular lysates are arrayed onto a nitrocellulose substrate and probed with a primary antibody. In turn, a biotinylated secondary antibody recognizes the presence of the primary antibody. The biotinyl groups are then detected by streptavidin linked to reporter molecules such as quantum dots or enzymes such as HRP that carry out catalyzed reporter deposition for signal amplification. (b): Schematic representation of biotinylated albumin bound to a substrate and detected by a streptavidin-quantum dot.

Fig 2.

Comparison of the autofluorescence of nitrocellulose coated glass slides and silicon generated using 254 nm wavelength excitation. Silicon has minimal autofluorescence when imaged using a fluorescence imager. After 5 minutes of imaging, the signal from the nitrocellulose coated glass slides on the left is very intense. The silicon substrates on the right, in contrast, appear to yield little if any intrinsic signal. It should be noted that silicon does not exhibit any fluorescence at any wavelength.

Fig. 3. Surface morphology of roughened silicon. (a) Scanning electron microscopic image showing a top view of 20 min reactive ion etched silicon. (b) Idem, 180 min etch, identical magnification. The dotted scale bars represents 200 μm . (c) Scanning electron microscopic image of cross-sectional view of 180 min reactive ion etched silicon. The dotted scale bar represents 100 μm .

Fig. 4. Comparison of untreated, blank silicon and 20 min RIE etched, MPTMS treated silicon substrates. The physicochemically modified silicon substrate has increased signal, confirming previous data.

Fig. 5.

Comparison of 180 min RIE etched MPTMS and APTES treated silicon substrates with a nitrocellulose-coated substrate. The latter yields no interpretable data due to phantom spotting and a high background signal.

Fig. 6. Graph of average spot intensity vs. biotinylated albumin (g/mL) for different physicochemical silicon substrates. NB: Both axes are logarithmic.

Fig. 7.

Comparison of blank silicon, 20 and 180 min RIE etched MPTMS and 180 min etched APTES treated silicon substrates with a nitrocellulose-coated substrate in a reverse-phase protein microarray setting. Please note that the nitrocellulose substrate was scanned with a gain of 35dB, whereas the other substrates were scanned at 70dB. The nitrocellulose substrate whites out at higher gains than 35 dB, viz. Fig 1.

Fig 8. Short (repeated) dilution series of biotinylated albumin in PBS, T-PER/SDS/β-mercaptoethanol and urea/CHAPS indicating that the solvent has a large influence on the spot morphology on a 20 min etched MPTMS treated silicon substrate.