Passivation of aluminum with alkyl phosphonic acids for biochip applications

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Abstract
Self-assembly of decylphosphonic acid (DPA) and octadecylphosphonic acid (ODPA) was studied on aluminum films using XPS, ToF-SIMS and surface wettability. Modified aluminum films were tested for passivation against silanization and subsequent oligonucleotide attachment. Passivation ratios of at least 450:1 compared to unprotected aluminum were obtained, as quantified by attachment of radio-labeled oligos.

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1. Introduction
During the last two decades, the use of self-assembled monolayers (SAM’s) in the production of well-defined surfaces has undergone tremendous growth, advanced in part through the depth of surface characterization methods [1,2]. SAM’s are ordered molecular assemblies that are spontaneously formed by the adsorption of molecules with head groups that show affinity to a specific substrate, which enables ordering on the surface without any preassembly [2].

One of the most active areas of research with SAM’s is towards the production of biochips. The fabrication and manipulation of molecular assemblies, combined with molecular recognition and computational chemistry to elucidate structure–function relationships, forms the core of modern surface chemistry. One of the important design aspects for biochips is to isolate probing molecules into active areas of transduction. This paper discusses the development of a biosensor array for ultimate use in molecular diagnostics. The role of SAM’s to form chemically distinct regions on the patterned aluminum surface of a biochip is explored.

In the literature, alkanethiols on gold [3–5] and alkylsilanes on silica [6] are the two most extensively studied classes of SAM’s. Recent literature has also seen an array of functionalities developed that can be used with more active metals. Studies have been reported for titanium [7–10], tantalum [11–14] and aluminum [15,16] oxides coated with alkylsilanes, thiols, phosphonates, carboxylic acids, and so forth. In this paper, the aluminum oxide and phosphonic acid system will be explored. Aluminum, being a light metal, bears potential in a broad range of technological applications. Aluminum can be classified as an active metal due to its tendency to oxidize quickly. The aluminum oxide layer is chemically bound to the surface, and it seals the core aluminum from any further reaction.

Aluminum has been used in the development of biosensors due to its dielectric properties supporting, for example, the propagation and localization of surface plasmons. These biosensors are fabricated by patterning an aluminum layer on top of a glass/quartz substrate, where the pattern often takes the form of microscopic holes [17,18]. An aluminum layer can also be patterned macroscopically by opening “windows” to the underlying substrate that precisely define the location and morphology of capture zones [19]. For DNA-based biosensors on glass, the primary anchoring molecules are silanes. For development of a similar biochip with aluminum as the metallic component, it is desirable to prevent attachment of molecular recognition functionality (silane) to the metal film.

A selective and localized passivation approach is needed for these kinds of mixed material biochips. The most commonly used sensors are based upon glass in conjunction with gold. Bare aluminum poses a problem as silane attaches to both aluminum (Al–O–Si) and glass (Si–O–Si). Hence prior to silanization, aluminum needs to be passivated. Alkyl phosphonic acids are reported to passivate a variety of metal oxides, such as titanium or aluminum oxide, while not binding to SiO2 surfaces in an aqueous medium [18,20,21]. This represents a potential approach for targeted molecular placement of capture oligos on patterned aluminum film biochips.
One of the main reasons for using phosphonic acids rather than the well-known carboxylic acids is their stronger binding with the oxide [22,23]. Aluminum forms a native oxide when exposed to an oxygen-containing environment. The phosphonic acids are mostly deposited from an organic or water-diluted (10^–3 mol/l) solution [1]. It is known that the phosphonic acids interact with the aluminum hydroxyl groups, where an increase in the number of surface hydroxyls enhances the phosphonic acid deposition [24,25]. Phosphonic acid specifically reacts to hydrated aluminum oxide, through the Al–O–P bond. The Si–O–P bond formed on glass substrates is easily hydrolysable [20]. The phosphonic acid prevents subsequent chemical treatments, such as exposure to silane containing molecules, from reacting with the aluminum. Further, capture molecules can be attached to non-aluminum surfaces via reaction with a specific functional group of the silane. However, tailoring a surface through the attachment of phosphonic acid SAM’s requires careful aluminum surface preparation prior to monolayer self-assembly [24]. The main issue related to the use of the aluminum–phosphonic acid system is the question of whether a dense SAM can be formed. Although for many systems it has been proven that the chain length is a crucial factor in the formation of a SAM [23], it has been shown that this is not the case for phosphonic acids [26,27]. Relatively well-oriented and stable films have been formed using pentaphosphonic acid [26] and octylphosphonic acid [1].

In this paper, we describe the preparation and characterization of self-assembled monolayers of alkyphosphonic acids (primarily octadecyl phosphonic acid and decyl phosphonic acid) on an aluminum oxide surface. As described previously, this method has been extensively used for purposes of corrosion protection. This paper explores the potential of the alkyl phosphonic acid layer to be used as an agent to improve selective silanization of sensors based on metallic array structures. The other aspect of this study will be to investigate the role played by alkyl chain length of phosphonic acid in formation of an effective passivation layers towards silanization. This study further characterizes passivation against immobilization of oligonucleotide molecules as a step towards development of DNA-based diagnostic biochips.

2. Experimental

2.1. Chemicals

All chemicals are reagent grade. The phosphonic acids employed were n-butylphosphonic acid—CH_2(CH_2)_3PO(OH)$_2$ (BPA), n-decylphosphonic acid—CH_2(CH_2)$_3$PO(OH)$_2$ (DPA) and n-octadecylphosphonic acid—CH_2(CH_2)$_7$PO(OH)$_2$ (ODPA). They were purchased from Alfa Aesar (purity 98%). 3-Glycidoxypropyltrimethoxysilane (GPS). The oven was sealed, pumped down, and purged three times with ultrapure nitrogen. After 8 h, the oven was purged with nitrogen and the substrates were removed.

2.2. Substrate preparation

Mostek Inc. (Provo, UT) provided plain glass substrates and substrates coated with 100 nm aluminum films. Windows in Al were fabricated with conventional photolithography: the Al substrates were coated with a positive photoresist then soft baked. A photomask with an array of 200 μm × 200 μm square holes with a periodicity of 400 μm was generated using an Electronmax MM250 pattern generator. An Electronic Visions EV-420 mask aligner was used to transfer the pattern onto the photoresist. After developing the photoresist, the exposed Al areas were etched out with a standard Al-etchant and the photoresist was removed.

The substrates were cleaned using solvent wash. The wash included acetone, isopropyl alcohol and methanol. After solvent wash, samples were rinsed with doubly distilled water (ddH$_2$O) and dried using nitrogen. This was followed by oxygen plasma cleaning using a Harrick plasma cleaner. The plasma cleaner was operated at the medium power setting (200 W) for 5 min. At this point, the samples were placed in a closed petri dish and set aside for 30 min before any further processing.

2.3. Self-assembly

Phosphonic acid solutions of 1 mM were prepared in methanol, a concentration that is normally reported in the literature as the molecules behave as free species in the solution. The passivation layer was self-assembled onto the substrates by soaking them in phosphonic acid solution at room temperature for 48 h. Samples were cleaned in methanol and dried under nitrogen. After passivation, samples were annealed under nitrogen for 4 h at 90°C. The physisorbed phosphonic acid was removed using triple methanol and water washes.

2.4. Silanization

After cleaning, the substrates were placed in a Fisher Scientific oven at 115°C with a small vial containing 1.5 ml of 3-glycidoxypropyltrimethoxysilane (GPS). The oven was sealed, pumped down, and purged three times with ultrapure nitrogen. After 8 h, the oven was purged with nitrogen and the substrates were removed.

2.5. Surface characterization

Surface wettability was investigated by measuring the contact angle in a sessile water drop experiment. A water drop of 1 ml volume was used in each measurement. Three independent readings were taken for each sample.

The thicknesses were measured by reflectance variable angle spectroscopic ellipsometry (VASE) using material databases for underlying films (Al on glass, Al$_2$O$_3$). The measurements were carried out in the spectral range of 300–1000 nm. The measured $\Psi$ and $\Delta$ for the phosphonic acid layer were fitted using “thickness” as the fitting parameter. The fitting procedure was repeated for measurements at various positions on the sample surface to get the average thickness for each film.

XPS analyses were performed on an Axis Ultra spectrometer from Kratos (Manchester, U.K.) equipped with a concentric hemispherical analyzer and using a mono-chromatized aluminum anode X-ray source maintained at 15 KeV. The samples were investigated under ultrahigh vacuum conditions: 10$^{-8}$ to 10$^{-7}$ Pa. Samples were analyzed with a pass energy of 160.0 eV for survey scans and 40.0 eV for high energy resolution elemental scans.

Static ToF-SIMS (Cameca/ION-TOF IV SIMS) was performed with a monoisotopic 25 keV $^{65}$Ga$^+$ primary ion source. The primary ion (target) current was typically 2 pA, and the raster area of the beam was 500 μm × 500 μm. The negative ion spectra were calibrated using H$^-$, O$^-$, OH$^-$, CH$_2^-$, CH$_3^-$ and C$_2$H$_4$.

2.6. Radio-labeling

Probe oligonucleotides were 3’-end-labeled with [α-$^32$P]dATP using a Terminal Transferase labeling kit. The reaction mixture consisted of 5 pmol of 5’ end amine-terminated oligonucleotide, 5 μl of 10× NE buffer 4, 5 μl of 2.5 mM CoCl$_2$, 0.5 μl Terminal Transferase
(20 units/µl), 0.5 µl of 10 mM dATP [α-32P] and ddH2O to a final volume of 50 µl. The mixture was incubated at 37°C for 30 min. 10 µl of 0.2 M EDTA (pH 8.0) was added to terminate the reaction. The products were purified using sephadex g25 columns. The purified product was spiked with 245 pmol of unlabelled amine-terminated oligonucleotide. The solution was dried using a speed vac. Dried oligonucleotide was re-suspended in 150 mM phosphate buffer (pH 8.5). The silanized substrates were spotted with 1 µl of 50, 5, 0.5 and 0.05 µM solution of oligonucleotide, covering three orders of magnitude. After spotting, the substrates were held at room temperature in a humid chamber for at least 4 h. The substrates were then rinsed with ddH2O and blown dry with N2. These substrates were scanned using a phosphor-screen.

3. Results and discussion

Contact angles on cleaned aluminum and glass substrates were nearly zero. Water droplets completely wetted these surfaces. AFM scans of the cleaned Al surfaces showed about 1.2 nm surface roughness, which is low enough so as not to affect contact angle [28]. After surface treatment, the aluminum surfaces became hydrophobic. The contact angle observed for BPA coated aluminum was 57 ± 2°. DPA-coated aluminum was 103 ± 2° and that for ODPA was 116 ± 2°. These values correspond well with the lengths of the alkyl chains of these molecules, with slight aberration in the case of DPA [29,30]. The contact angle on GPS-silanized glass substrates was 57 ± 2°. Nearly the same values were observed with similarly silanized aluminum substrates: 59 ± 2°.

Average SAM thickness was calculated based on ellipsometry measurements. The phosphonic acid monolayer is modeled as a stack of Al/Aluminum oxide/ODPA (or DPA). The aluminum oxide film thickness was obtained from ‘Ψ’ and ‘Δ’ measured on the reference aluminum oxide/Al stack by using the optical properties from the standard database (1.75–1.80 in the visible spectrum), in which the only unknown was the thickness of the oxide layer. The thickness of the oxide layer was deduced from fitting ‘Ψ’ and ‘Δ’ obtained on three spots of this reference film. This value was used to represent the thickness of the oxide layer in the optical stacks of Al/aluminum oxide/ODPA (and DPA) for the ‘Ψ’ and ‘Δ’ data collected from three spots on the ODPA and DPA sample.

We adopted the Tauc–Lorentz (TL) generalized oscillator dispersion model to obtain dielectric properties and thicknesses of the phosphonic acid (PA) monolayers [31]. The TL model combines the Tauc joint density of states and a single transition Lorentz oscillator to account for interband absorption and bound electron absorption, respectively. The dielectric function ε1 + iε2 and photon spectrum are used in the TL dispersion model.

In the TL model the monolayer thickness was one of the fitting parameters and the dispersion of ‘n’ and ‘k’ were calculated from that of the dielectric function by ε1 + iε2 = [(n + ik)^2]. The average aluminum oxide layer thickness was measured to be 3.77 ± 0.02 nm. The average ODPA monolayer thickness was measured to be 1.73 ± 0.24 nm, with a thickness 0.61 ± 0.06 nm for DPA. Similar thickness measurements were reported for monolayer formation of both ODPA and DPA films [32]. Refractive indices (n) in the visible range were 1.82–1.90 for ODPA and 1.62–1.70 for DPA. There was no measurable absorbance (k) in all cases.

To further analyze the films, XPS characterization was performed to determine the chemical identities of the surfaces. The surface chemical composition of clean, unmodified Al was 49.0 at.% Al, 41.5 at.% O, and 8.5 at.% C, with fluorine accounting for 1 at.% or less. Fluorine was present on the aluminum substrates and could not be removed using an oxygen plasma cleaning. Based on high-resolution XPS scans of F 1s and C 1s, the fluorine present in the samples were not in form of fluorocarbons. A high-resolution XPS scan around the Al 2p peak confirmed the presence of an oxide layer, as shown in Fig. 1.

The Al 2p spectrum was resolved into metallic and oxide components by fitting in the 65–85 eV binding energy region. The fitted spectrum illustrates the presence of an oxide peak at the binding energy of 75.9 eV as well as an Al metal peak at 72.9 eV. This agrees well with the binding energy separation of 2.8 eV reported in an XPS-spectra handbook. The presence of oxide on the Al surface is required for the Al–O–P bond formation.

Fig. 2 shows the survey spectra for ODPA modified aluminum (Fig. 2a) and unmodified aluminum (Fig. 2b). The phosphorus 2s and 2p peaks are indicative of the modification of the Al surface by ODPA. Also, C 1s peak suggests the formation of phosphonic acid layer on the aluminum film. These peaks are absent on glass after similarly treating it with ODPA. High-resolution spectra were collected at the O 1s, C 1s, P 2p and Al 2p peaks (Table 1).

The C/P ratio for DPA is 10.6. This is close to the theoretical value of 10. The C/P ratio for ODPA is 21. That number is greater than the theoretical value of 18 is most likely due to attenuation of the P photoelectrons through the moderately thick hydrocarbon over-layer. Adventitious carbon may also contribute somewhat to this ratio, although its contribution is probably small because of the hydrophobic nature of the surface. Similar trends have been reported in other studies when complete coverage was observed [25].

The stability of these monolayer films was evaluated by dipping them in both methanol and water for 24 h and then evaluating the surface coverage. Neither the water contact angles, nor the surface chemical compositions, changed on the ODPA and DPA modified aluminum films. This was not the case for the BPA coated substrates.

Table 1 Atomic percentages (±2.5% relative error) were calculated from high-resolution XPS scans.

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<thead>
<tr>
<th></th>
<th>O 1s</th>
<th>C 1s</th>
<th>P 2p</th>
<th>Al 2p</th>
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<tbody>
<tr>
<td>Al + DPA</td>
<td>28.5</td>
<td>28.5</td>
<td>2.7</td>
<td>39.4</td>
</tr>
<tr>
<td>Al + ODPA</td>
<td>21.7</td>
<td>51.8</td>
<td>2.5</td>
<td>23.5</td>
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Fig. 2. Survey spectra across the binding energy range 0–600 eV. (a) XPS-spectra of ODPA modified Al. (b) XPS-spectra of bare Al.

Their contact angles decreased from 81° to 45°. This behavior is also corroborated in the Al/P ratio from the XPS study. The Al/P ratio for ODPA is 9.5, for DPA is 14.7 and for BPA is 18.2. The smaller ratio is suggestive of a dense phosphonic acid film. Thus ODPA forms the most dense film among the phosphonic acids used in this study. The samples passivated with BPA had the highest Al/P ratio of 18.2 among the phosphonic acids used in this study. This explains the instability of the BPA film. Studies have shown that the alkyl chain length has a strong influence on the molecular packing during self-assembly: the longer the chain length, the better the orientation of the molecules on the surfaces. The longer chains are better able to self-assemble due to an increase in van der Waals (vdW) attractive forces with increasing chain length, because the strength of the vDW interactions per adsorbate is proportional to the number of methylene units in the adsorbate [2,33].

The resistance of the ODPA and DPA films to silanization with GPS was evaluated by testing the silanized films for Si using XPS. The resulting high-resolution narrow scans over the Si 2s and Si 2p regions were at background levels, indicating that there was no silane detectable on the surface.

Complementary ToF-SIMS measurements were then performed to demonstrate the selective functionalization of the aluminum and glass regions on a prototype biochip. Square windows (200 μm × 200 μm) were opened in 100 nm aluminum films on a glass substrate. These substrates were passivated with DPA or ODPA and then silanized with GPS. Positive secondary ion spectra failed to show any characteristic peak for the modified aluminum surface that was easily distinguishable from the glass region. This was due to hydrocarbon peaks from the alkyl chains of both the phosphonic acids and the silane, where the lower water contact angle (higher surface free energy) of the silanized glass region would also make it more susceptible to contamination from adventitious hydrocarbons. However, characteristic peaks were observed in the negative ion spectra for the phosphonic acids and the silane. Two fragmentation peaks of the phosphonic acid group: PO$_2^-$ (AMU 63), and PO$_3^-$ (AMU 79), confirmed the presence of phosphonic acid on the Al surface. In the case of GPS, a quantifiable peak was observed at AMU 75. There were two possible sources for AMU 75 ion: AlO$_3^-$ and SiCO$_2$H$_3^-$. A negative ion image scan across a raster area of 500 μm × 500 μm showed the localization of AMU 75 in the glass region, confirming the identity of the ion as SiCO$_2$H$_3^-$. The PO$_2^-$ (AMU 63) and PO$_3^-$ (AMU 79) (Fig. 3a) images were localized to the aluminum regions on the substrate. The phosphate ion fragment peaks signals were almost down to the background level in the case of glass. This confirms the selective formation of the alkyl phosphonate layer on aluminum acting as a passivation layer against the silane.

Radio-labeling experiments showed the effectiveness of the passivation layer against oligonucleotide adsorption. The four spots on glass in Fig. 4A are the four serial dilution spots, corresponding to:...
Fig. 4. Radio-labeled oligonucleotide spots on silanized slides. The black spots correspond to spotted oligonucleotide probe. Lanes: A—glass, B—aluminum, C—ODPA passivated aluminum, D—DPA passivated aluminum. All the substrates have been silanized with GPS.

(top to bottom) to a serially diluted oligonucleotide at a level of 103 ± 10, 85 ± 12, 37 ± 12 and 19 ± 5 fmols. The spots on aluminum (Fig. 4B) correspond to 187 ± 14, 72 ± 25, 57 ± 12, 24 ± 7 fmols.

The slides of phosphonic acid modified aluminum (Fig. 4C and D) show passivation towards silanization and oligonucleotide adsorption; the spots are not visible on the DPA and ODPA modified surfaces. The grey region around the highest concentration spot on the DPA modified surface suggests a slightly higher background as compared with ODPA modified film. The background levels from radio-labeled oligonucleotide on ODPA passivated correspond to a concentration of 0.4 fmols. This result shows a passivation ratio of at least 450:1 when using DPA passivation. The background levels on DPA passivated slides were higher, especially near the higher concentration spotted region. The background levels on DPA correspond to a concentration of 0.9 fmols. This result shows a passivation ratio of at least 200:1 when using DPA passivation. The BPA modified film was not stable in an aqueous medium; hence it was not considered a suitable candidate for passivation studies.

4. Conclusion

In conclusion, phosphonic acid modification is an important step in the development of biochips consisting of patterned aluminum/glass surfaces. In addition, this passivation can work on other metal oxides such as titanium oxide and tantalum oxide. Passivation is critical for reducing the background signal to low enough levels in order to allow effective biochip operation.

References