Derivatization of pillared silicon substrates using poly(ethylene glycol) and 1-dodecanethiol

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Abstract: Surfaces that exhibit hydrophobic and hydrophilic characteristics are essential when developing Lab-on-Chip technologies. The availability of a droplet to be maneuvered on a surface develops from hydrophobic surface characteristics, while non-protein fouling characteristics are found in hydrophilic surfaces. By combining these traits, an ideal surface can be produced for biomedical applications. To satisfy these surface requirements, we have attached 8-arm hydroxyl-terminated poly(ethylene glycol) to silicon oxide and methyl-terminated 1-dodecanethiol to gold. By applying these techniques to a pillared substrate the result is hydrophilic poly(ethylene glycol) covalently bonded to pillar tops and hydrophobic 1-dodecanethiol bound to pillar sides and troughs. This creates a surface where the hydrophobic pillar sides and troughs suspend a droplet for movement while the hydrophilic pillar tops prevent loss of essential proteins.

1. INTRODUCTION

Protein fouling, the adsorption of proteins on a surface, typically occurs at the solid/liquid interface of a surface exposed to an aqueous protein solution. This causes many problems in the development of biomedical devices for areas in biotechnology and especially Lab-on-Chip technologies. Hydrophobic and hydrophilic surfaces have been shown to affect protein adsorption differently [1,4,6] and would therefore aid in the development of these devices. It is known that protein fouling occurs more readily on hydrophobic surfaces [1, 4] and that hydrophilic surfaces are generally non-fouling [1,4,10,11]. The ability to utilize both hydrophobic and hydrophilic characteristics in a surface provides an efficient strategy to maneuver fluids and minimize protein fouling which is essential when dealing with Lab-on-Chip developments.

Whitesides and others have revealed that alkanethiols, such as 1-dodecanethiol (DDT), can be assembled directly onto gold in the presence of an assembly solution yielding similar results [3-9] to produce tightly packed self-assembled monolayers (SAMs). The utilization of hydrophobic terminal groups, such as methyl groups, in these SAMs results in hydrophobic surface characteristics. It has also been proven that poly(ethylene glycol) (PEG) can be covalently attached to silicon oxide resulting in a hydrophilic non-fouling surface using different techniques [2,10,11].

The goal of this work was to create a model surface that exhibits the non-fouling characteristics of a hydrophilic surface, to prevent loss of proteins, with the hydrophobic characteristics that allow a droplet to be maneuvered on a surface. PEG modified surfaces were generated on silica oxide during a three step process to obtain a hydrophilic surface. Hydrophobic surfaces were achieved through DDT attachment to gold surfaces using SAM architecture. The hydrophilic PEG modified surfaces on silicon oxide and hydrophobic surface of SAMs made with DDT attached to the gold surface were analyzed using contact angle measurements and Electron Spectroscopy for Chemical Analysis (ESCA).

2. MATERIALS AND METHODS

2.1 PEG attachment to silicon oxide

Glass slides (VWR micro cover glass 24x50 mm, VWR International) were immersed in a piranha solution (1:3 (v/v) ratio of H2O2 and concentrated H2SO4) for 15 min and rinsed with DI water three times for cleaning and creation of additional hydroxyl groups on the surface. Slides were immediately dried under nitrogen, placed in a Teflon holder, and submerged in a 20% (v/v) 1,6-hexamethylene diisocyanate (HDI) (Cat# D124702, Sigma-Aldrich, St. Louis, MO) in tetrahydrofuran (THF) (Cat# T5267, Sigma-Aldrich) with 1% wt dibutyltin dilaurate (Cat# 291234, Sigma-Aldrich) as a catalyst for 45 min
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Substrates derivatized with HDI were then rinsed in fresh THF to remove unbounded HDI and reacted with dendritic (8-arms) hydroxyl-functionalized PEG having MW 10,000 Da (Cat# 0J000L08, Nektar, Huntsville, AL) in a 10% (w/v) solution of THF in a presence of 1% wt dibutyltin dilaurate for 45 min. All surface manipulations were conducted under dry nitrogen inside of a reactor. Substrates were then washed with fresh THF to remove all non-covalently bonded dendritic hydroxyl-functionalized PEG, dried with nitrogen, and sealed under nitrogen. The procedure was also completed using N, N-dimethylacetamide (DMAc) (Cat# D5511, Sigma-Aldrich) as a solvent and a comparison was made. All chemicals were used as received.

2.2.1 Dodecanethiol attachment to gold

Single sided gold substrates were sonicated for 3 min in acetone (Cat# 650501, Sigma-Aldrich) and methanol (Cat# M3641, Sigma-Aldrich). Substrates were then placed in DDT (Cat# 471364, Sigma-Aldrich) solution at 1 mM total concentration in assembly solvent (Ethanol 200 proof, Aperr QAstock) for 20-24 h, sealed under argon and wrapped in aluminum foil. Substrates were later rinsed three times for 30 sec on each side with ethanol, dried with argon, and sealed under argon.

2.3 Pillared substrate preparation

Pillared substrates were used with pillar top dimensions 23.9-51 μm and pillar trough dimensions 47.1-67.7 μm with silicon oxide on pillar tops and gold coated pillar sides and troughs. DDT was covalently attached to gold using above procedure. It was discovered that any disruption to the pillared surface during handling would result in an inability to proceed with both dendritic hydroxyl-functionalized PEG treatment and DDT treatments. This study therefore only considers pillared substrates after DDT treatment.

2.4 Contact angle measurement

Water contact angles were acquired through use of a goniometer. Advancing contact angles were measured by placing a 5μl droplet on the substrate surface, taking a photograph and using FTR200 Dynamic Contact Angle Analyzer to measure left and right side contact angles at the water/surface interface. Six spots per sample were measured and the average was documented [Table 1].

Table 1 Substrate Contact Angles.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>43.05</td>
</tr>
<tr>
<td>Glass with piranha</td>
<td>37.65</td>
</tr>
<tr>
<td>Glass with PEG</td>
<td>52.54</td>
</tr>
<tr>
<td>Glass with PEG and DDT</td>
<td>49.74</td>
</tr>
<tr>
<td>Gold</td>
<td>78.40</td>
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<tr>
<td>Gold with PEG</td>
<td>76.76</td>
</tr>
<tr>
<td>Gold with PEG and DDT</td>
<td>98.54</td>
</tr>
<tr>
<td>Gold with DDT</td>
<td>99.93</td>
</tr>
</tbody>
</table>

2.5 Electron Spectroscopy for Chemical Analysis measurement

All ESCA spectra were taken on a Surface Science Instruments S-probe spectrometer. This instrument has a monochromatized Al Kα X-ray of spot size 800 μm. A pressure of less than 5x10⁻⁹ was held constant during readings. Pass energy for survey spectra (composition) was 150 eV, pass energy for HRC scans was 25eV and the take-off angle was 55º. All samples had three spots analyzed with a detailed sulfur reading taken for those exposed to DDT.

2.6 Fakir state

The relationship between surface roughness and Fakir state contact angle was utilized to determine the ability of a pillared surface to produce a water droplet in the “Fakir state” [12], which is

\[
\cos \Theta_f = -1 + (1 + \cos \Theta_{\text{silicon oxide}}) \Phi
\]

\[
\Phi = \frac{b^2}{(a+b)^2}
\]

Figure 1 Relationship between surface roughness and Fakir state contact angle.
essential for Lab-on-Chip applications. The ability to produce a droplet in the Fakir state was studied using 0.03 mg/ml fibrinogen solution, which simulates a 1% (v/v) plasma concentration. The relationship between pillar top and pillar trough size can also be used to determine the expected Fakir state contact angle [Figures 1 and 2].

Figure 2 Pillared substrates with a, b, and \( \Phi \) values and corresponding contact angles of pillared substrates with 1-dodecanethiol on pillar sides and troughs. \( \Theta_f \) = Fakir expected contact angle. \( \Theta \) = measured contact angle.
3. RESULTS AND DISCUSSION

To validate DDT attachment procedures contact angle measurements and ESCA analysis data were analyzed. Very minimal contact angle changes between gold substrates and gold substrates exposed to dendritic hydroxyl-functionalized PEG treatment were observed which indicates very little or no PEG attachment to gold substrates after THF sonication treatment. Also, minimal contact angle changes were detected between gold substrates exposed to PEG and DDT treatment vs. gold substrates with DDT treatment only [Table 1]. ESCA results also indicate, based on surface composition atomic percentage of gold, carbon, and sulfur that the dendritic hydroxyl-functionalized PEG process does not disturb the assembly of DDT on gold surfaces [Figure 3]. ESCA results confirm that DDT is present on the surface of the gold substrate as seen from a detailed sulfur scan; the presence of sulfur corresponds to DDT attachment [Figure 4]. This can be compared to the expected surface composition for DDT, 34.3% gold, 2.7% sulfur, and 63% carbon ± 1%. The average of three detailed sulfur scans for DDT on gold with the procedure described yielded 32.72% gold, 2.93% sulfur, and 64.35% carbon.

Validating the dendritic hydroxyl-functionalized PEG attachment procedure also utilized data from contact angle measurements and ESCA analysis. A decrease in contact angle after piranha treatment on silicon oxide substrates indicate additional hydroxyl groups added to silicon oxide surfaces [Table 1] before PEG treatment. ESCA results indicate dendritic hydroxyl-functionalized PEG covalently bound to silicon oxide when using THF as solvent [Figures 5 and 6] by the decrease of silicon surface atomic percentage from 24.1% to 12.1% and the increase of carbon surface atomic percentage from 17.3% to 42.8%.

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**Surface Composition for Gold Substrates**

![Surface Composition for Gold Substrates](image)

*Figure 3* Surface composition atomic percentage for gold substrate with DDT, gold substrate with dendritic hydroxyl-functionalized PEG and DDT, and expected surface composition for DDT on gold.
Figure 4 Detailed sulfur scan for gold substrate with DDT displaying sulfur peak, indicating the presence of DDT.

Silicon Oxide with PEG and Silicon Oxide with PEG and thiol

Figure 5 ESCA scan comparing silicon oxide vs. silicon oxide with dendritic hydroxyl-functionalized PEG and DDT with carbon and silicon surface composition atomic percentage averages over three test spots.
Figure 6 ESCA results indicating surface percentages of carbon, silicon, and oxygen on silicon oxide, silicon oxide with dendritic hydroxyl-functionalized PEG using DMAc as a solvent, and silicon oxide with dendritic hydroxyl-functionalized PEG using THF as a solvent.

Figure 7 Detailed sulfur scan of silicon oxide substrate treated with dendritic hydroxyl-functionalized PEG compared to silicon oxide with dendritic hydroxyl-functionalized PEG and DDT.
As seen in Figure 6, the surface composition of silicon oxide substrates before treatment compared to silicon substrates treated with dendritic hydroxyl-functionalized PEG in DMAc is relatively the same and therefore indicates no PEG attachment with this solvent. Also it can be shown through ESCA analysis that there is no sulfur present on the silicon oxide substrates exposed to DDT treatment, considering sulfur is an indication of DDT attachment to surfaces. This confirms that DDT does not bind to silicon oxide or disrupt the dendritic hydroxyl-functionalized PEG covalently bound to silicon oxide during the PEG treatment procedure [Figure 7].

We have found pillar dimensions that support droplets in the Fakir state with DDT present on pillar sides and troughs [Figures 1 and 2]. Further experiments will investigate the possibility of PEG attachment to pillar tops using the procedure described. Also, the ability of pillars exposed to PEG and DDT treatments to support droplets of 1% plasma in the Fakir state are being further investigated.

5. CONCLUSIONS
This work provides insight on creating hydrophilic and hydrophobic surfaces for Lab-on-Chip technologies. It has been found that dendritic hydroxyl-functionalized PEG can be successfully covalently bound to silicon oxide via an HDI tether using THF as a solvent, in the presence of additional hydroxyl groups created through piranha treatment. ESCA results have confirmed that dendritic hydroxyl-functionalized PEG does not attach to gold surfaces, or prevent the attachment of DDT to gold. DDT was found to covalently attach to gold, but not to silicon oxide surfaces. Also, it was shown that gold surface on pillar sides and troughs can be derivatized by the DDT attachment validating the potential to produce droplets in the Fakir state using a pillared substrate.

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