Wettability Changes Induced by Biochemical Surface Reactions

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We report the use of proteins, lipids, and enzymes for the preparation of surfaces with reversible wettability changes, in particular, surfaces capable of switching from hydrophobic to hydrophilic and back. We demonstrate that these reactions can be used for engineering capillary systems with gating properties.

Surfaces with stimuli-responsive changes in wettability are of interest for the development of micro- and nanofluidic sensors, switches, and gates. Smart surfaces with significant and reversible changes in contact angles induced by light,[1−4] temperature,[5,6] electric potential,[7−10] and solvents[11,12] have been reported.

The high specificity and mild reaction conditions of enzymatic reactions are attractive features in surface engineering. In this work, we report the preparation of surfaces with wettability changes using reactions of the enzymatic cleavage of lipid- and protein-rich surfaces. Our main focus was to develop surfaces that can reversibly switch from hydrophobic to hydrophilic. The underlying principle is illustrated in Figure 1. The adsorption of amphiphilic compounds such as lipids or proteins on hydrophobic substrates reverses the polarity of the surface, producing a hydrophilic surface that is wettable by water. Enzymatic cleavage of the hydrophilic adlayers restores the original hydrophobicity of the surface. These reactions can be repeated in cycles, which, coupled with porous surfaces, offers a capillary system with gating properties. For wettable surfaces (contact angle $< 90°$, $\cos\theta > 0$), water is sucked up in the pores and, under gravity, runs through the filter. For nonwettable surfaces (contact angle $> 90°$, $\cos\theta < 0$), drops of water roll off the surface as excess pressure is needed to overcome the capillary pressure and push water into the pores.[13]

Reactions were investigated with two substrates: Si wafers and porous glass filters ($R_{pore} \sim 50\ \mu$). Si wafers were mainly used for the ellipsometry, contact angle, and spectral characterization of the surfaces, while filters were primarily used for the demonstration of gating properties. Given the similarity of glass and oxidized Si wafer surfaces, we assumed that the structure and properties of the surfaces supported on these two silicas were similar. Hydrophobization of both silica substrates was performed through the reaction with *n*-octadecyldimethyl (*N*,*N*-dimethylamino)silane[14,15] to yield covalently attached monolayers (CAMs) of the C_{18} groups: C_{18}-wafers and C_{18}-filters, respectively. The ellipsometric thickness of the C_{18} CAMs on wafers was 1.3 ± 0.1 nm. This is ∼50% of the length of fully stretched C_{18} chains, indicating monomolecular surfaces of disordered alkyls.[15,16] The dynamic contact angles (adv/rec) of the C_{18}-wafers indicated hydrophobic surfaces: $105/95 \pm 1°$ (water) and $34/31 \pm 1°$ (hexadecane). For the C_{18}-filters, water did not penetrate them; drops rolled off when the filter was tilted.

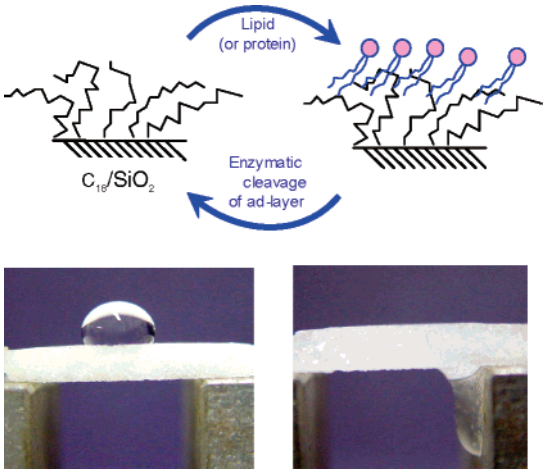

Figure 1. Adsorption of amphiphilic compounds (lipids or proteins) on hydrophobic porous filters (left) produced hydrophilic surfaces (right), through which water runs freely. Enzymatic cleavage of the adsorbed layers restores the original hydrophobic surfaces (left).

Two biospecific reactions have been investigated: (1) the cleavage of adsorbed phospholipids by phospholipase and (2) the cleavage of adsorbed bovine serum albumin (BSA) by trypsin. More experimental details are provided as Supporting Information.

BSA−Trypsin System. This section describes the wettability and thickness changes upon adsorption of protein (BSA) on the C_{18} surfaces and the subsequent reactions with trypsin. The adsorption of BSA on the C_{18}-wafers greatly improved their wetting by water. The best results were obtained with BSA solutions of 25 mg/mL: water contact angles dropped to $50/40 \pm 5°$ (adv/rec). The thickness of the BSA layer was 3 ± 0.3 nm, suggesting a single-layer adsorption of protein (Figure 2). For

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(13) The capillary pressure is obtained as $\Delta P = 2 \cdot \gamma \cdot \cos\theta / R$ (cylindrical pores). If the pore radius $R = 50\ \mu$, the contact angle $\theta = 100°$, and the surface tension $\gamma = 72$ mJ/m^2 (water), $\Delta P = 500$ Pa ≈ 4 Torr (∼55 mm of water).

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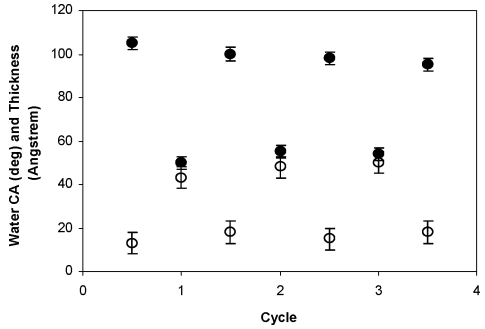

Figure 2. Advancing water contact angles (closed symbols) and thickness (open symbols) for the adsorption of BSA on the C_{18}-wafers followed by the reaction with trypsin. Data for three consecutive cycles are shown.

solutions with low concentrations of BSA (10 mg/mL), the adsorption was low (thickness $\sim$ 0.5 nm), and the water contact angles decreased insignificantly (90/70 $\pm$ 5° (adv/rec)). For solutions with high concentrations of BSA (50 mg/mL), contact angles initially dropped to $\sim$30°, but then rapidly (within minutes) increased to 70/50 $\pm$ 5° (adv/rec). For C_{18}-filters, the adsorption of BSA (25 mg/mL) produced hydrophilic surfaces, which water ran through freely. The infrared spectra of the BSA-coated wafers were consistent with the presence of protein on the surface. No removal of BSA (by IR, ellipsometry, and contact angles) was observed after rinsing the BSA$-C_{18}$-wafers with water or the buffer solution used for the adsorption (phosphate buffer, pH 8).

After the incubation of BSA-coated wafers with trypsin solution (37 °C, 3 h), hydrophobic surfaces were obtained. We believe that trypsin cuts the adsorbed BSA into smaller pieces that do not adsorb strongly to the C_{18} surface and are washed away by water. Figure 2 shows the contact angle and thickness data for three consecutive cycles of BSA adsorption followed by incubation with trypsin. Although the switching between hydrophobic and hydrophilic surfaces was reproducible, the hydrophobic surface was not fully restored. Water contact angles of the C_{18}-wafer slightly decreased (several degrees), and the thickness increased ($\sim$0.2 nm) with each cycle. This suggests an incomplete removal of amphiphilic compounds and/or nonspecific adsorption of the enzyme on the C_{18} surface. We found that the repeatability of the cycles can be greatly improved by rinsing the surfaces with ionic surfactants after the reaction with the enzyme. The activity of trypsin toward adsorbed BSA allows for some speculation regarding the orientation of protein molecules on the surface. Trypsin cuts the C-terminal side of hydrophilic, basic residues (lysine and arginine) located on the surface of a protein molecule.[17] It has been reported that no additional cleavage sites were exposed on BSA upon adsorption, even though the protein had lost much of its native structure.[18] Furthermore, for BSA adsorbed on a hydrophobic surface,[18] trypsin preferentially cuts versus chymotrypsin, which is known to cut hydrophobic residues. Thus, our data supports the conclusions of previous work[18] that BSA adsorbed onto hydrophobic surfaces with the hydrophilic portion of the molecule facing away, and the hydrophobic portion toward the surface.

Lipid−Lipase System. This section describes contact angles and thickness changes due to the adsorption of phospholipids on the C_{18} surfaces followed by their enzymatic cleavage with lipase. Two lipids were investigated: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanol-amine and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. The deposition of lipids was done using lipid vesicles, and the results obtained for both lipids were similar. The lipid-coated C_{18}-wafers showed water contact angles of 36/25 $\pm$ 2° (adv/rec) and a thickness in the range of 1.7−2.2 nm. The lipid-coated C_{18}-filters were hydrophilic, and water ran freely through them. The thickness and the low contact angles of the lipid-coated C_{18} suggested that the lipid molecules formed a monomolecular layer with their polar headgroups facing away from the hydrophobic substrate. According to the infrared spectra, alkyl chains in the adsorbed lipids were ordered. The positions of the CH_2 stretchings ($\nu_a \sim 2918$ and $\nu_s \sim 2848$ cm^{-1}) showed a high degree of order, similar to that reported for closely packed monolayers of long-chain alkyls.[19] It is noted that the lipids adsorbed to the C_{18} surfaces rather strongly: no removal of lipid layers (by IR, ellipsometry, and contact angles) was observed after washing the surfaces with pure water or buffer solutions.

The enzymatic cleavage of the adsorbed lipids was done through incubation (37 °C, 3 h) with PDAse I, the enzyme that cleaves the P−O−C bonds from the phosphate headgroup of the lipid to the hydrocarbon portion of the molecule.[20] The reactions of lipid-coated C_{18}-wafers with PDAse I gave hydrophobic surfaces with water contact angles of 102/93 $\pm$ 5° (adv/rec) and a thickness of 1.4 $\pm$ 0.1 nm, which is close to the values for the original C_{18} surfaces. The lipid-coated filters, after incubation with PDAse I, turned hydrophobic; water could not run through the pores.

In conclusion, we described the use of proteins, lipids, and enzymes for the preparation of surfaces with reversible wettability changes, in particular, surfaces capable of switching reversibly from hydrophobic to hydrophilic. We demonstrated that these reactions can be used for the engineering of capillary systems with gating properties.

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Supporting Information Available: Experimental details on the preparation and characterization of the surfaces. This material is available free of charge via the Internet at http://pubs.acs.org.

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