The Princess and the Pea:
The Persistence of Surface Chemistry Effects on Multi-Layer Biomolecule Adsorption

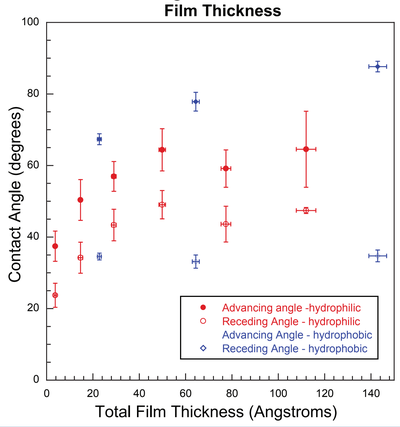
Susanna Michael, Jasmine Wallas, K. T. Queeney

Department of Chemistry, Smith College, Northampton MA 01063

Introduction

Biofilms are aggregations of proteins and polysaccharides that stick to surfaces. In the Queeney lab, we have been adsorbing the protein poly-l-lysine (PLL) and the polysaccharide alginate to functionalized silica. We use a hydroxyl-terminated hydrophilic silica and a silane-terminated hydrophobic silica, and these surface differences cause the polylysine and alginate to adsorb differently. This in turn produces measurable differences in the film thickness of the layers as well as the wettability of the surface. Because we know that the starting surface has a large impact on how the biofilms are initially adsorbed,[1] we are curious to find if there is a point at which the nature of the initial surface stops having an effect on the wettability of the sample.

Contact Angle on PLL versus Total Film Thickness



Poly-L-Lysine (Above)
Contact angles for PLL on hydrophilic surfaces appear to be leveling off around 60º (advancing) by the 5th or 6th bilayer, but the contact angles for the hydrophobic surface continue to increase.

Alginate (Right)
The contact angle for alginate on hydrophilic surfaces appears to have leveled off around about 30º. The third bilayer of the hydrophobic sample has reached a similar value; this suggests a limiting value for a relatively continuous alginate film.

Multi-Layer Adsorption

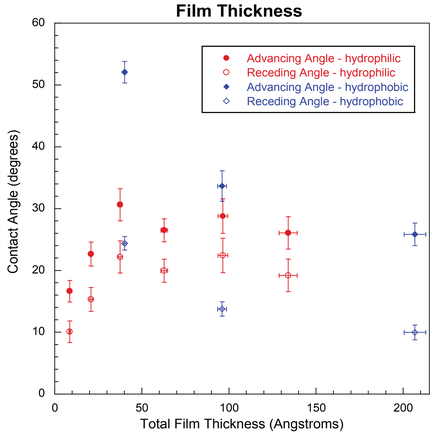
Poly-l-lysine and alginate are adsorbed in alternating layers to a functionalized silica surface. A layer of PLL followed by a layer of alginate is referred to as a bilayer. A total of three bilayers were adsorbed to the hydrophobic surface, while six bilayers were adsorbed to the hydrophilic surface.

Experimental Procedure

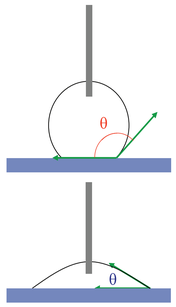
Hydrophilic
Si(100) wafers were P-Cleaned (4:1 H_2O_2: H_2SO_4). 0.5 M KBr/PLL (2 μ g/mL) was adsorbed for 60 minutes. Alginate (2 μ g/mL) was adsorbed for 60 minutes. The adsorption process was repeated until six bilayers were adsorbed to the wafer. Contact angle and ellipsometry measurements were taken after each layer was adsorbed.

Hydrophobic
Si(100) wafers were silanized with octyldimethylchlorosilane. PLL (2 μ g/mL) in pH 11 buffer (2 mL:18 mL) was adsorbed for 30 minutes. Alginate (2 μ g/mL) was adsorbed for 60 minutes. The adsorption process was repeated until three bilayers were adsorbed to the wafer. Contact angle and ellipsometry measurements were taken after each layer.

Contact Angle on Alginate versus Total Film Thickness



Dynamic Contact Angle Goniometry



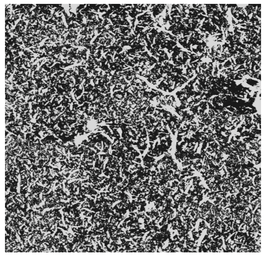
The contact angle of a probe liquid on a surface reflects the surface free energy of the interface between the liquid droplet and the surface, and between the interfaces of both liquid and surface with air. A large contact angle indicates a non-wetting interaction, while a small contact angle indicates wetting of the surface by the probe liquid.

In dynamic contact angle goniometry we measure the contact angle of the drop as it advances across the surface; this is the advancing angle, θ_A. Because advancing drops tend to get "pinned" by non-wetting entities on a chemically heterogeneous surface, θ_A is most sensitive to hydrophobic (in the case of H_2O probe liquid) regions of the surface. We also measure the receding angle as the drop retracts, θ_R; this tends to register only the most wetting parts of the surface, where the drop clings as it recedes.

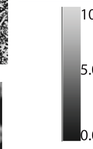
Conclusions

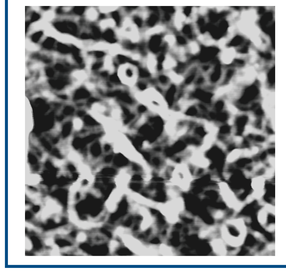
Even with six bilayers, the biofilm adsorbed on the hydrophilic surface has still not reached a thickness to match the third bilayer of the hydrophobic surface. Essentially, the starting nature of the silica is able to influence the adsorption about one hundred and twenty Angstroms away from the surface.

The AFM images show that the surface of the hydrophilic sample is much rougher than the hydrophilic surface. This is consistent with the contact angle data, which shows a higher contact angle hysteresis (difference between the advancing angle and receding angle) on the hydrophobic surface than on the hydrophilic surface.



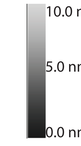
AFM image (10 μ m square) of the third bilayer of the hydrophobic sample, showing surface topography.

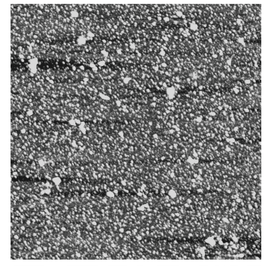




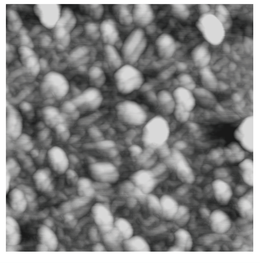
AFM image (1 μm square) of the third bilayer of the hydrophobic surface (zoom of above image.)

AFM image (1μ m square) of the third bilayer of the hydrophilic sample, showing surface topography.





AFM image (1 μ m square) of the third bilayer of the hydrophilic surface (zoom of above image.)



Acknowledgements

This work was supported by the National Science Foundation and by the Smith College STRIDE program.

References

1) Brown, J. E. 2008. A. B. Honors thesis, Smith College, Northampton, Massachusetts , United States.

2) Ganzelves, R. A.; Cohen Stuart, M.A.; van Vliet, T.; de Jongh, H. *Food Hydrocolloids* **2006**, *20,* 872-878.

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