

SAMS AND BIOFUNCTIONAL SURFACES: THE "INERT SURFACE" PROBLEM

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Introduction

Self-assembled monolayers (SAMs) of alkanethiols on gold and silver provide a technology that is uniquely suited for studying the interactions of biomolecules with surfaces.¹⁻³ When alkanethiols with the general structure of HS(CH₂)_nX self assemble onto surfaces of gold or silver, they form an ordered monolayer. The metal-sulfur bond anchors the SAMs on the surface, and the X groups point out and into the solution, and comprise the exposed surface. The macroscopic properties of SAMs can be engineered by changing the terminal groups at X. SAMs make it possible to control the composition and properties of the surfaces perpendicular to the plane of the solid support; soft lithography makes it possible to pattern the film in the plane;⁴ surface plasmon resonance (SPR) and quartz crystal microbalance can be used to measure interactions of molecules in solution with groups on the surface; electrochemistry is increasingly useful in modifying these surfaces.^{5,6}

One of the most fundamental problems in the studies of biosurfaces is how to make surfaces *resist* non-specific adsorption of proteins, since proteins adsorb onto most types of surfaces.⁷ We call surfaces that resist non-specific adsorption of proteins "inert surfaces". SAMs that present certain chemical groups make good inert surfaces. We use these SAMs in studies that require well-defined surfaces, such as biochemical analysis and the characterization of adhesion and migration of mammalian cells.¹

Experimental Techniques

We used SPR to quantify the amount of adsorbed proteins on surfaces. SPR is an optical method that allows in-situ measurement of the amount of protein bound on surfaces of SAMs on gold and silver films.^{1,2}

Studies with mammalian cells used either primary isolates from animals-- for example, bovine capillary endothelial (BCE) cells -- or transformed cell lines like NIH 3T3 fibroblast cells. Cells were cultured on SAMs under the same conditions as normal cell culture.^{8,9}

Results and Discussion

A series of SAMs with different X groups were used to test how the wettabilities of surfaces (characterized by the contact angle of water on the surface of each SAM) correlated with the amount of protein adsorbed on the surface (Fig. 1). Generally, hydrophobic surfaces tend to adsorb more protein; the correlation between wettability of the surface and the amount of adsorbed proteins is regular but variable from protein to protein, and there are notable exceptions. In particular, SAMs formed by oligoethyleneglycol (OEG)-terminated alkanethiols (whose hydrophobicity is intermediate) make the surface completely inert.¹⁰ These compounds thus follow no correlation between wettability of the surface and adsorption of protein.

In order to study how biomolecules interact with SAMs having different X groups, we required a range of these X groups drawn from a number of different structural classes. Many types of chemical reactions have been used to introduce functional group onto the surface of SAMs. There are two general approaches for covalent attachment of different functional groups: i) synthesis of the required thiols that carry the appropriate functional groups,⁸ and ii) introduction of functional groups after the formation of the SAMs, using chemistry such as the inter-chain anhydride method.¹¹ These two methods each have their advantages and disadvantages. We have found the anhydride method to be the most convenient for exploratory work.¹¹

Using the anhydride method, we have introduced various X groups on the surface of SAMs and screened their abilities to resist the non-specific adsorption of proteins on surfaces using SPR. We found that, in addition to OEG-terminated alkanethiols, several other types of chemical groups make SAMs inert. There are some common characteristics of

these chemical functionalities: they are electrically neutral, polar and hydrogen bond donors.¹¹

Why, mechanistically, some SAMs are inert is not clearly established. It is clear, however that there is a layer of water closest to the surface of SAMs that plays an important role in resisting the adsorption of proteins. Grunze and coworkers used experimental and theoretical approaches to address this problem in OEG-terminated SAMs.^{12,13}

Hydrophobic interactions between proteins and surfaces is one of the main driving forces for adsorption of proteins. We have used mixed SAMs of OEG-terminated thiols and CH₃-, C(C₆H₅)₃-, CH(C₆H₅)₂-, and CH₂(C₆H₅)-terminated thiols to dissect the relationship between the adsorption of protein and the shapes and densities of the hydrophobic patches on the surface (Figure 2).¹⁴

It is possible to combine SAMs with microcontact printing to define the shapes and sizes of adhesive islands on the surface to pattern proteins and cells. We have used this technology to pattern various types of cells. Discreet control of the sizes and shapes of cells allow cell biologists to examine questions that are difficult to address with conventional, commercial plastic or glass substrates. For example, using a combination of patterned surfaces, we determined that it is the overall *projection* area of a cell, rather than the *total attached* area of a cell, that is important in a cell's decision to survive or undergo programmed cell death.¹⁵ Figure 3 illustrates an example of making geometrically patterned surfaces to confine the attachment of cells. We have also used these technologies to investigate other questions concerning adherent cells using SAMs.^{9,16,17}

Recently, we and others have been able to control the adhesion of cells in culture dynamically. (Figure 4) Using electrochemistry, it is possible to release cells from their patterns of confinement,^{5,6} or to pattern several types of cells.¹⁸ Complementary methods derived from techniques in soft lithography and other means of microfabrication have been used to pattern different types of cells.^{19,20}

Conclusion

Alkanethiolate SAMs on gold and other metals are good tools with which to study biological phenomena at water-solid interfaces. Inert SAMs are particularly useful systems to study ligand binding and biofouling of the surface. SAMs also provide new tools with which to study fundamental issues in cell biology and tissue engineering. The exact reason of why some SAMs are inert is not known, although there is evidence that hydrophobicity of the SAMs and a thin layer of water close to the surface of SAMs may explain why some SAMs are inert.

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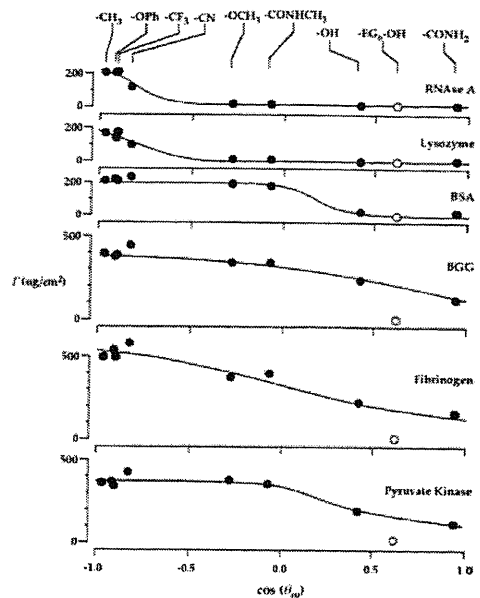


Figure 1. Surface density of adsorbed films of six proteins (Γ) on a variety of SAMs as a function of the contact angle of water on the SAM under cyclooctane ($\cos\theta_{c0}$). Values of Γ were obtained from SPR.

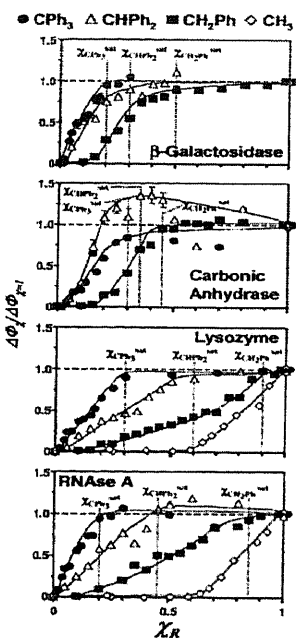


Figure 2. Amounts of protein adsorbed on mixed SAMs of $\text{HS}(\text{CH}_2)_{11}(\text{EG})_6\text{OR}$ and $\text{HS}(\text{CH}_2)_{11}(\text{EG})_3\text{OH}$ (where $\text{R} = \text{CPh}_3$, CHPh_2 , and CH_2Ph). Plots of $\Delta\Phi / \Delta\Phi_{\text{max}}$ (a measure of the amount of protein adsorbed normalized to the amount adsorbed at $\chi_R = 1$, χ is the relative percentage of $\text{HS}(\text{CH}_2)_{11}(\text{EG})_6\text{OR}$ in the mixed SAM) for the adsorption of galactosidase, carbonic anhydrase, lysozyme, and RNase A. We also plot the values of $\chi_R = 1$ with mixed SAMs of $\text{HS}(\text{CH}_2)_{11}(\text{EG})_3\text{OH}$ and $\text{HS}(\text{CH}_2)_{10}\text{CH}_3$.

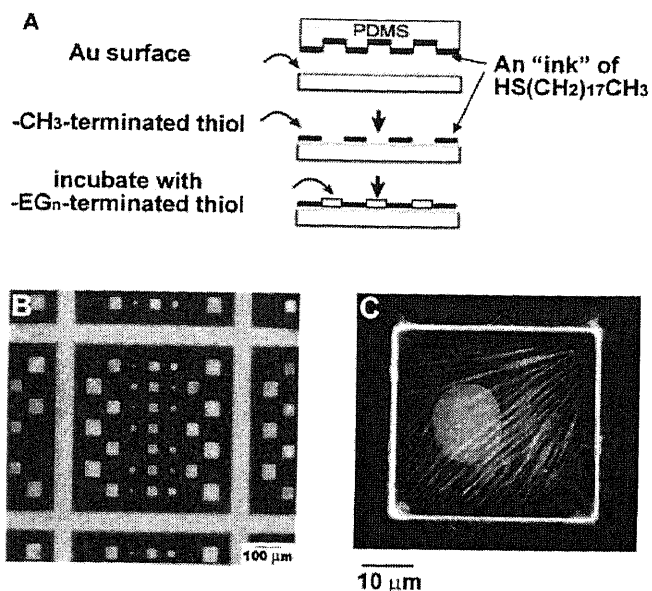


Figure 3. Application of inert surfaces: patterning of proteins and cells. (A) Using micro-contact printing to generate patterned surfaces. (B) Patterning of an extracellular protein fibronectin on SAMs. (C) Patterned SAMs enable the precise control of the geometry and area of a single BCE cell on the surface.

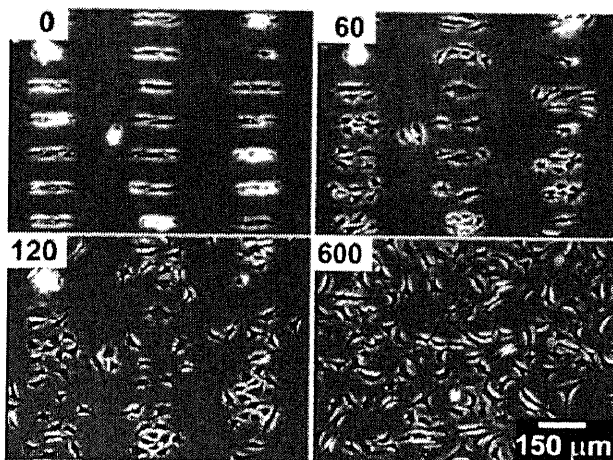


Figure 4. BCE cells were allowed to attach to a surface patterned with $\text{HS}(\text{CH}_2)_{11}(\text{EG})_6\text{OH}$ and $\text{HS}(\text{CH}_2)_{17}\text{CH}_3$. Application of a cathodic voltage pulse (-1.2 V for 30 s in this case) desorbs the $\text{HS}(\text{CH}_2)_{11}(\text{EG})_6\text{OH}$, thus releasing the cells from the microislands. The numbers indicate the time elapsed (in minutes) after the voltage pulse.