Protein Adhesion on SAM Coated Semiconductor Wafers: Hydrophobic Versus Hydrophilic Surfaces

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Abstract

The interface of biology and semiconductor materials has become an important topic of interest as researchers are merging living organisms with microelectromechanical systems (MEMS) in the pursuit of new microfabricated biomedical devices. Biological cells interface with the semiconductor surface through a host of interactions that are mediated by protein adhesion and film formation. To better understand this interface, we have conducted protein adsorption studies of a model system using bovine serum albumin (BSA) and compared it to the adsorption from a complex protein mixture of cell growth serum on uncoated and self-assembled monolayer (SAM) coated silicon wafers. Several characterization techniques - AFM, ellipsometry, water contact angle, and fluorescence microscopy - were used to evaluate the protein-adsorbed layer. An uncoated silicon surface was most attractive to proteins, forming a 70 Å thick film from a solution of BSA at a concentration comparable to that in cell growth serum. Coating with the hydrophobic octadecyltrimethoxysilane (OTMS) SAM reduced protein adhesion by ~15%. In contrast, a hydrophilic N-(triethoxysilylpropyl)-O-polyethyleneoxide urethane (TESP) SAM inhibited protein adhesion by greater than 50%. Protein adhesion studies with cell growth sera containing complex mixtures of proteins paralleled the BSA adsorption studies, clearly identifying the TESP coated surface as a promising biocompatible coating.

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Introduction

The interface of biology and man-made materials is an important topic of research that impacts a vast range of issues from barnacles on cargo ship hulls to medical implants (*1,2,3*). Biofouling of filter membranes (*4,5*), tissue compatibility of surgical implants (*6,7*), and cellular interfacing with semiconductor materials (*8,9,10,11*) are just some of the areas of study where biology and synthetic materials and devices are melding. In particular, the developing area of biological materials interfaced with micro-electromechanical systems (MEMS), or BioMEMS (*12,13*), has brought on considerable interest in how cells and other biological materials interface with semiconductor materials. Spontaneous adhesion of proteins and cells occur at the semiconductor surface through non-specific as well as specific interactions. The adhesion of proteins often precedes the adhesion of cells to the surface due to concentration and the kinetics of surface adsorption. Cells also excrete proteins (e.g., fibronectic, vitronectin) to prepare surfaces for adhesion (*14,15*). Hence, the most fundamental aspect of the biocompatibility of materials starts with the study of protein adsorption on surfaces.

Protein adsorption on a material is highly dependent upon the material's surface free energy and functionality (*16,17,18*). Numerous studies have been conducted to passivate material surfaces to cell and protein adhesion using simple hydrophobic and hydrophilic polymers and thin films. Grafted polymers and self-assembled monolayers are methods used to introduce functionality to surfaces, as well as establishing a packing density on the surface and specific orientation of the functionality. Polyethylene glycol (PEG), or polyethyleneoxide (PEO), functionalized surfaces are examples of hydrophilic coatings that offer excellent resistance to protein and cellular adhesion as both grafted polymer and SAM films (*19,20,21,22,23,24*). In contrast, hydrophobic surfaces, such as octadecyltrichlorosilane SAMs, tend to adsorb proteins as a monolayer thick biofilm (*25,26,27*). The current understanding of the difference in protein adhesion is related to the interfacial free energy of the coating in water (surface hydrophobicity), steric repulsion of the functionalized surface against protein (entropy losses in chain conformation and solvent expulsion), and conformational changes allowed by the adsorbing protein (*28,29,30*).

Surface passivation of semiconductors to biological materials is of particular interest in Sandia's development of microscale devices for cellular analysis. The Biocavity Laser project has developed a compact flow cell that rapidly analyzes populations of cells for disease or malignancy (*31,32*). The flow channel acts as a laser cavity that is optically pumped at just below the lazing threshold. As the cells pass through the light beam, the cell's shape and internal contents, which vary from healthy to diseased individuals, modulate the light, simultaneously bringing it above the lazing threshold and altering the spectral transmission. Hundreds of thousands of cells can be analyzed within minutes, generating histograms of data that distinguish the cell population as healthy or diseased.

The Biocavity Laser is affected by biofouling of the microchannels from the cell solution, resulting in impeded flow. As cells pass through the flow channels they tend to aggregate at the channel exit causing increased flow restriction with time. As a result, the operational time of the device spans only several minutes. Adhesion of the cells, whether they be normal human astrocytes, blood cells (red or white), or glioblastomas, is highly dependent upon the surface coating (*33*). The adhesion of cells, however, is most likely preceded and dictated by the adsorption of proteins that are part of the cellular serum. A study of protein adhesion to coated surfaces should provide information on how to tailor the chemistry of surfaces to inhibit or modify protein adhesion and/or denaturation, ultimately tuning cellular adhesion.

In this report, we describe the preparation of two SAM coatings, one hydrophilic and the other hydrophobic, on silicon wafers and characterize their affinities towards proteins in cellular sera. The hydrophilic SAM was prepared from a polyethylene glycol silane agent and the hydrophobic SAM prepared from a hydrocarbon silane. A model study of protein adhesion was first performed using bovine serum albumin (BSA), providing a means to comparably analyze surface adsorption experiments of complex protein mixtures in cell growth sera. The surfaces before and after protein adsorption were characterized using water contact angle measurements to assess changes in surface hydrophilicity, AFM to monitor protein adsorption at the nano-scale, fluorescence microscopy to optically identify the adsorbed medium, and ellipsometry to measure average film thickness with varying time and conditions.

Experimental

General. Silane reagents were obtained from Gelest, Inc. OTMS was distilled under reduced pressure prior to use. TESP was used as received as a mixture of 4 – 6 mers of oligoethylene glycol (90% purity). The Si(100) substrates were single-side polished (1 Å level roughness) with a 40 Å thick oxide surface. Water was purified through a Barnstead Type D4700 NANOpure Analytical Deionization System. Solvents used were obtained from Fisher Scientific and were of reagent grade unless otherwise noted.

SAM coatings. Self-assembled monolayers (SAMs) were produced of n-octadecyltrimethoxysilane (OTMS) and N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP) on 1 cm^2 silicon substrates. In preparation for monolayer deposition, all wafers were carried through the same cleaning steps: The wafers were first submerged in a Piranha etch solution (1:1 conc. H_2SO_4/30% aqueous H_2O_2) for 10 minutes at 100 °C to remove traces of adsorbed organic material. The wafers were then rinsed judiciously with deionized water, followed by two washes in isopropyl alcohol, allowing them to soak in baths for 10 minutes each wash, then two successive 10 minute baths in anhydrous toluene. The wafers were then removed and dried for protein adhesion studies on bare silicon, or immediately placed into a SAM solution.

TESP or OTMS SAM films were deposited onto the wafers at room temperature using n-butylamine as a catalyst. For OTMS deposition, a stock solution of 50 mM OTMS was prepared by adding 1.06 mL of OTMS to 50 mL of anhydrous toluene (Aldrich) with 0.1 mL of n-butylamine. The TESP solution was prepared at a 12 mM concentration by mixing 0.27 mL of TESP and 25 μL of n-butylamine in 50 mL of anhydrous toluene. SAM deposition was performed by soaking the wafers in their respective silane solutions for 4 hours under ambient conditions in sealed jars. After removal from the solution, the newly deposited monolayer films were rinsed with anhydrous toluene, dried, and then heat treated overnight at 80°C in an Isotemp 281A oven (Fisher Scientific). Subsequently, the films were ultrasonicated for a few minutes in chloroform to clean the surface. The films were then dried and stored in a dessicator at < 10% humidity.

Protein adsorption. Bovine serum albumin (BSA) was prepared as a 0.1 mM (6.6 mg/mL) solution by dissolving 66 mg of BSA (Sigma) in 10 mL of MOPS buffer solution. The MOPS buffer solution was prepared by dissolving 2.1 g of 4-morpholinepropanesulfonic acid (MOPS) and 2.93 g of NaCl into 500 mL of deionized water, then adjusted to a pH of 7.4 with 10% aq. NaOH solution. Bare silicon, TESP and OTMS coated wafers were placed in 10 mL of freshly prepared BSA solution and incubated for 2 or 24 hours at room temperature. Upon removal from solution, the wafers were judiciously rinsed with deionized water from a squeeze bottle to remove any weakly bound protein and salt from the solution. This procedure was repeated using freshly coated wafers to determine standard deviations of each experiment (Tables 1 and 2). Fluorescein isothiocyanate-labeled BSA was incubated with the coated and uncoated wafers in an identical procedure as that described above. The films were air-dried and stored in the dark at 4 °C.

Protein adsorption studies were also performed on the SAM coated silicon substrates with cell culture growth sera. Astrocyte basal medium (Clonetics) with 10% fetal bovine serum (HyClone), used for normal human astrocyte (NHA) cell culturing, and nutrient mixture F-12 (Sigma) medium with 10% fetal bovine serum, used for glioblastoma (GBM) cell culturing, were used. Similar to the procedure above for BSA adsorption, OTMS and TESP coated wafers were incubated in a 10 mL quantity of either the NHA or GBM sera for 2 and 24 hours at room temperature. Upon removal from the solutions, the films were rinsed thoroughly with deionized water from a squeeze bottle. The films were air-dried and stored in the dark at 4 °C.

Contact Angle Measurement. Contact angle measurements were taken in air, by the sessile drop method, of all SAM films used both before and after exposure to protein. A Video Contact Angle System 2500 (Advanced Surface Technology, Billerica, MA) was used to capture static images of deionized water droplets on the wafer surface. Several areas on the wafer were sampled to obtain an average over the entire surface. The data are reported in Tables 2 – 4.

Atomic Force Microscopy (AFM). All SAM films were examined using AFM (Nanoscope IIIA, Digital Instruments, Santa Barbara, CA) before and after exposure to protein. All AFM imaging was performed in tapping mode, obtaining height and amplitude images. Images

were taken at several scan sizes ranging from 1 - 10 µm, at a rate of 2 Hz. Multiple areas on each film were examined, and representative images are shown in the Figures.

Fluorescence microscopy. Films exposed to fluorescein isothiocyanate-labeled BSA were examined using a Leitz Wetzlar (Germany) optical fluorescence microscope. The surfaces were illuminated with 450-480 nm light and emission wavelengths greater than 515 nm were amplified and viewed using a CCD72 camera system (MTI, Michigan City, IN). NIH image software (v. 1.61) was used to capture and average images from the camera's field of view for further analysis.

Ellipsometry. An AutoEL ellipsometer (Rudolph, Ledgewood, NJ) provided thickness measurements of the adsorbed protein layers on the coated and uncoated silicon wafers. A standard modeling method (*34*) allowed the determination of film thickness from the delta and psi values. Freshly coated SAM wafers were used as references. An incidence angle of 70° was used with a wavelength of 632.8 nm and an index of refraction of 1.54 for the protein layers.

Results and Discussion

SAM Preparation and Characterization

Commercially available silane coupling agents were used to prepare hydrophobic and hydrophilic SAMs on silicon wafers. The wafers were pretreated with a Piranha solution to clean the surface and to generate the maximum amount of surface exposed silanols. Piranha cleaning left the surface hydrophilic with a water contact angle of 40° (Table 2). SAM depositions on the cleaned and dried wafers were performed in organic solvents under ambient conditions. The silane-coupling agents used in this work were trialkoxysilanes. The reasoning for using trialkoxysilanes instead of the more commonly used trichlorosilanes was reproducibility of the deposited film. Trichlorosilane-based SAMs, although rapid in formation on silicon surfaces, were difficult to control with regard to reproducibility of film quality. The high reactivity of the Si-Cl bond to hydrolysis makes the trichlorosilanes highly sensitive to trace quantities of water in the organic solvents, as well as the adsorbed water on the wafer surface. Too little water and the SAM formation can be incomplete. Too much water, however, will produce aggregates of the silanes in solution that subsequently bind to the substrate, resulting in a rough surface. Good quality SAMs result from a low siloxane polymerization rate in solution relative to SAM formation on the substrate. Trialkoxysilanes, with their relatively slow hydrolysis rates in solution, are excellent reagents for high quality SAM formation on silicon surfaces, exhibiting little sensitivity to the amount of trace water content in solution (*35,36*).

Hydrophobic and hydrophilic SAMs were prepared using the silane agents octadecyltrimethoxysilane (OTMS) and N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP), respectively. SAM depositions for both agents were performed in toluene with N-butylamine as a catalyst. The trialkoxysilanes required one to several hours to form a complete

SAM. In comparison, trichlorosilane coupling agents typically require less than one hour. The trialkoxysilanes yielded reproducible SAM films with homogeneous and full coverage over the silicon substrate.

While OTMS is a pure compound and forms a highly ordered, crystalline-like monolayer film (*37,38*), TESP contains a mixture of PEO oligomers, between 4 – 6mers, incapable of producing highly packed films with crystalline-like nature. Figure 1 shows a schematic of the types of films that are produced on silicon substrates from these two silanes. AFM images of the OTMS and TESP SAMs are shown in Figure 2. As expected, OTMS produced a homogeneously smooth SAM film with a rms roughness of 1 Å compared to the TESP SAM that gave an rms roughness of 13 Å. The films are, for the most part, homogeneously flat. The images of Figure 2 were taken of areas where aggregates were present to allow for some contrast. Films prepared with TESP generally had more aggregates present on the surface compared to the OTMS films. Water contact angle measurements of the OTMS and TESP films (Table 2) are consistent with SAMs prepared from octadecyltrichlorosilane and a 4 – 6mer oligoethylene glycol grafted silane, respectively (*19,22,26*).
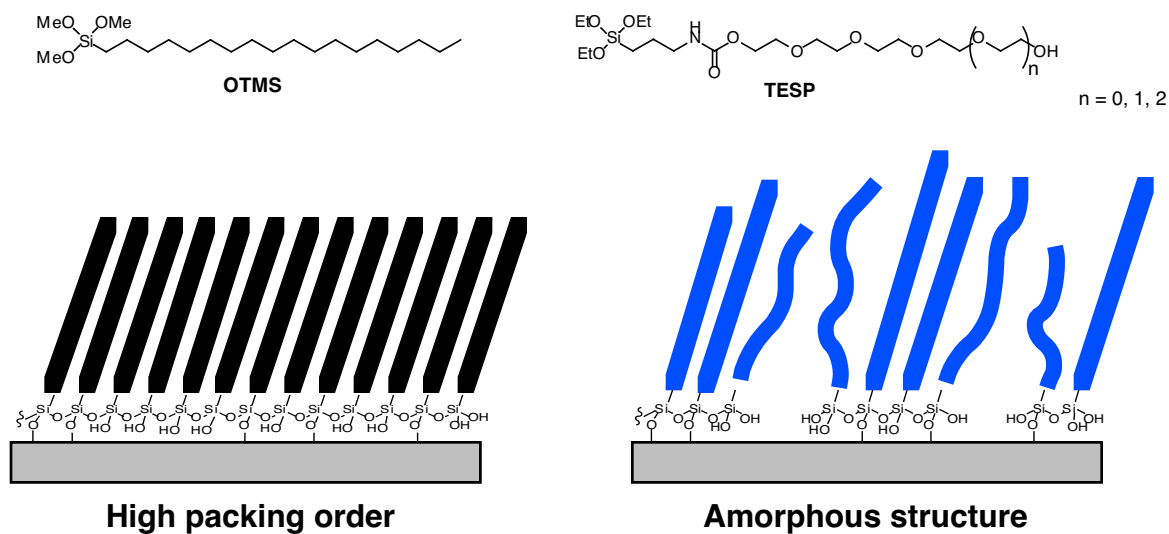

Figure 1. Octadecyltrimethoxysilane (OTMS) (left) and N-(triethoxysilylpropyl)-O-polyethylene oxide (TESP) urethane (right) chemical structures and schematics of their SAM structures on silicon.
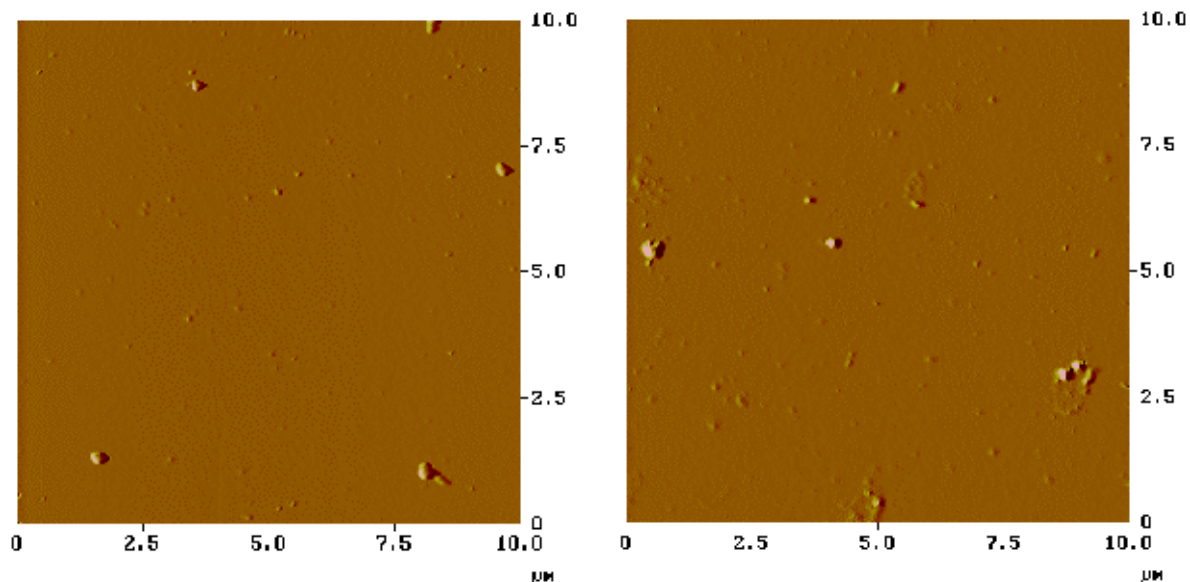
Figure 2. AFM images of OTMS (left) and TESP (right) SAMs on silicon wafers. Areas with surface adsorbed aggregates are shown to allow for some contrast.

BSA Adsorption on SAM Coated Silicon

Adhesion of proteins to surfaces is a complex phenomenon involving multiple interactions that occur between the surface and protein, as well as the reorganization of interactions that occur within the protein itself following surface contact. Proteins are coiled linear polymers that rearrange their tertiary shape in response to polar and hydrophobic interactions within their environment. Although it is difficult to fully understand the collective forces that drive the adsorption of proteins and the formation of protein-based biofilms on surfaces, simple model systems can be a guide for the analysis of biofilm formation. In this section, we will investigate the interactions of a model protein, bovine serum albumin (BSA), with coated and uncoated silicon wafers. This model system will establish the relative quantity of adsorbed proteins to surfaces and their characteristic surface features. The model data will then aid in elucidating observed features and film characteristics of surface adsorbed proteins from complex solutions.

Serum albumins (39,40) are the most abundant protein in the circulatory system of vertebrate organisms. Their apparent role is that of a transport vehicle for numerous ligands, ranging from fatty acids to metal ions, throughout the body. Serum albumins have multiple receptor sites for ligands as diverse as aspirin, Au(I), bilirubin, Cu(II), and diazepam, just to name a few. The protein carries a net negative charge (-17) at physiological pH with a heart-shaped tertiary structure. A crystal structure of serum albumin is shown in Figure 3 (41). The long axis of the protein is ~ 80Å and the width is ~ 30Å. The protein is conformationally flexible but at the same time robust to heat, surviving temperatures up to 60 °C for 10 hours. Serum albumins share high

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sequence homologies between mammalian species. For example, BSA and human serum albumin (HSA) share about 76% homology.

Figure 3. Structure of serum albumin.

ODTS and TESP SAM coated silicon wafers were exposed to 0.1 mM (6.6 mg/mL) solutions of BSA (66 kD) in MOPS buffer at pH 7.4, for 2 and 24 hours at room temperature. The concentration of BSA is similar to the concentration of proteins in cell culture sera (~6 mg/mL), which in turn is near physiological protein concentrations (~40 mg/mL). Protein adhesion studies found in the literature (*23,24,26,27,29*) have typically used concentrations between 1.0 – 0.01 mg/mL, which is the concentration range where the protein-surface adsorption isotherm rise is steepest. By employing concentrations at which the adsorption isotherm has the steepest slope, the effect of surface passivation is more distinct. In the present studies, we explore conditions comparable to those used in cell adhesion studies where the protein concentrations are several fold to orders of magnitude higher, well above the saturation point of protein adsorption on surfaces. Long surface exposure times were also employed to ensure that the protein-surface interactions were equilibrated.

Figure 4 shows AFM images of OTMS and TESP coated silicon surfaces after 24 hours of incubation with the BSA solution. The protein-adsorbed surfaces had rms roughness of 27 Å on the OTMS surface and 24 Å on the TESP surface. Both surfaces were covered with a fairly uniform coating of protein, with larger aggregates dispersed sparsely over the surface. On the OTMS surface, areas with fractal-like structures of the adsorbed protein film were frequently observed (Figure 5). Such images are consistent with those found in the literature for serum albumin adsorption onto octadecyltrichlorosilane SAMs on silicon (*26*). As a control, OTMS and TESP coated wafers were also incubated in buffered solution in the absence of BSA to identify

structural changes that might occur in the coating. AFM imaging revealed no changes in the films' structures or surface coverage after 24 hours of incubation time at room temperature.
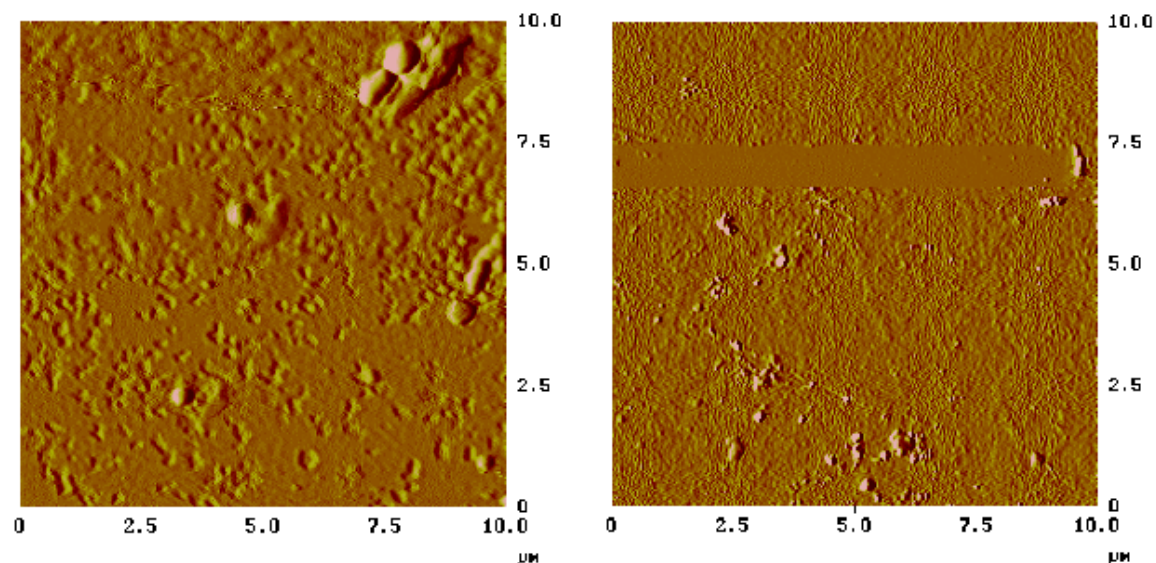
Figure 4. AFM images of OTMS (left) and TESP (right) coated silicon wafers after 24h incubation with BSA solution (0.1 mM).
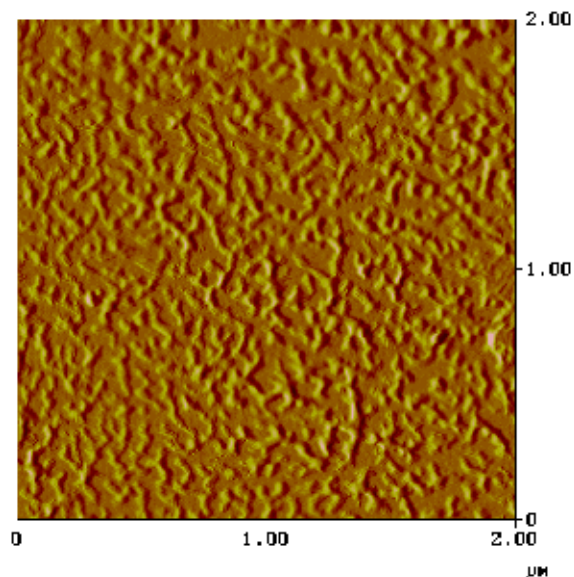
Figure 5. Fractal-like image of BSA on OTMS coated silicon observed with AFM.

Due to the high protein coverage, determination of the protein film thickness by AFM was inconclusive; instead, thicknesses were determined by ellipsometry. Table 1 lists the film thickness of adsorbed BSA on bare silicon and the two SAM coated surfaces after 2 and 24 hours of incubation time. On the TESP surface, BSA forms a 35 Å thick surface coating after 2 hours, but the film appears to lessen over time (20 Å) after 24 hours. This decrease in film thickness is

suggestive of protein desorption off the surface as the protein-surface interactions come to equilibrium. The OTMS film induces the formation of a fairly thick 50 Å coating of BSA that was stable over time. Bare silicon, however, was the most attractive surface for BSA, inducing the formation of a 62 Å thick film after 2 hours that increased to 70 Å after 24 hours.

      The asymmetrical shape of BSA makes the evaluation of the protein orientation on the SAM surface speculative. A 35 Å thick layer with full coverage over a surface, such as that observed with the TESP coating after 2 hours of BSA exposure, suggests a monolayer of the protein lying on its side. A previous study also reports a horizontal orientation of BSA on PEO-SAM coated surfaces with maintenance of the protein's globular structure (*24,42*). A protein coating of 70 Å thickness, on the other hand, could mean either a monolayer of protein has adsorbed in a nearly vertical orientation, or that a multilayer of highly tilted or denatured proteins forms the film. Protein denaturation on the surface would result in shrinkage of the protein-adsorbed film through the unraveling and flattening of the protein to the surface. Denaturation of surface bound protein can also result in desorption of proteins adjacent to the denaturing protein (*27*). The specific orientation of BSA on surfaces and the extent of protein denaturation were indeterminate within these experiments, and we shall limit our discussions to more general statements of protein adsorption with regard to adsorbed film thickness.

Table 1. Characterization of BSA adsorption on coated and uncoated Si

Coating	Film Thickness (Å)[a]		Protein aggregates /2500 μ^2 [b]
	2h w/ BSA	24h w/ BSA	
Bare Si	62 ± 0.2	70 ± 1.1	<1
OTMS	51 ± 18	50 ± 27	10
TESP	35 ± 15	20 ± 14	5

a) Measured by ellipsometry.
b) From fluorescence microscopic images. Aggregates measured were between 2 – 5 microns in diameter.

      Water contact angles of the initial and post-BSA treated surfaces are consistent with the film thickness measurements. Adsorption of a protein, which is amphiphilic, to hydrophilic bare silicon and TESP coated surfaces will increase surface hydrophobicity, resulting in an increase in contact angle. The hydrophobic surface, on the other hand, will become somewhat more hydrophilic with the addition of polar residues to its surface. The BSA film thickness loss observed on the TESP surface, going from 2 hours to 24 hours of incubation (Table 1), is reflected in a decrease of contact angle, again suggestive of protein loss from the surface

      To confirm the adsorption of BSA on the coated surfaces, a fluorescent-labeled derivative of BSA was used and the protein bound films imaged with fluorescence microscopy. Microscopic images are shown in Figure 6, and the densities of adsorbed protein aggregates are reported in Table 1. The spectral intensity from the thin film of adsorbed protein, observed to be covering the surface uniformly by AFM, could not be measured via this technique. The instrumentation did,

however, allow a crude measurement of protein adsorption from the density of observable large aggregate, fluorescent structures (i.e., > 2 microns in size). The data reported in Table 1 were taken from areas in the microscopic images that were void of aggregates larger than 5 microns, which may have some influence over the adsorption of proteins within their vicinity as well as dominate the image contrast against nearby smaller proteins. Overall, the TESP coated surface had less fluorescent material bound to the surface compared to the OTMS coated surface, consistent with the AFM images. Bare silicon, however, which was observed by AFM to be heavily covered with BSA, did not show any fluorescence in this experiment. Apparently, silicon, with its absorption maximum near 480 nm, efficiently quenches the fluorescence of fluorescein (*43,44*). Even the fluorescence from protein aggregates, visibly present by AFM, was quenched. The SAM films must act as spacers between the fluorophores and silicon reducing the energy transfer and allowing the proteins, at least from the aggregates, to fluoresce. A thicker oxide layer on the silicon wafer should offer a better platform for future fluorescence studies of fluorescent-tagged BSA.

Table 2. Contact angle of coated and uncoated Si before and after BSA adsorption

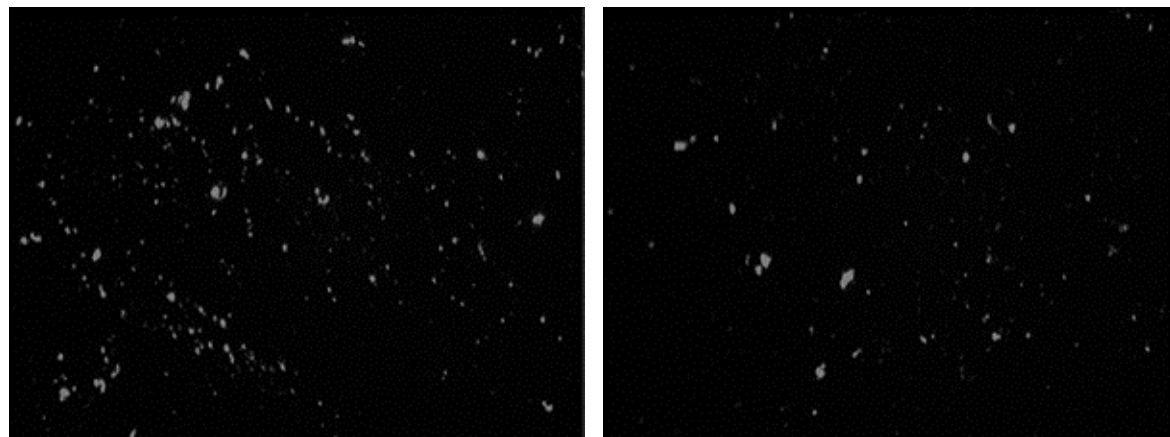| Coating	Water Contact Angle		
	Initial	2h w/ BSA	24h w/ BSA
Bare Si	40°	73°	71°
OTMS	109°	86°	84°
TESP	55°	70°	67°

Figure 6. Fluorescence microscope images of OTMS (left) and TESP (right) coated silicon wafers after 24 hours of incubation with fluorescein-tagged BSA solution. Image sizes are 400 microns across.

Protein Adsorption from Cell Growth Sera

Cell growth sera are sterile media composed of complex mixtures of proteins, antibiotics, growth hormones, and amino acids. Cell adhesion studies on solid substrates are often conducted in the presence of such media, but the interaction of proteins to the substrate surface is often overlooked. Protein adhesion and denaturation can completely alter the substrate's characteristics, with regard to surface polarity, functionality, and morphology. The adhesion and proliferation of cells on a surface is then dictated not only by the initial surface coating but also by the subsequent layer of coating formed by proteins from solution (*14, 45*).

The interactions of two cell growth sera with the hydrophilic TESP and hydrophobic OTMS SAMs were examined with AFM, water contact angle, and ellipsometry. The media were standard sera for growing normal human astrocytes (NHA) and glioblastoma (GBM, cancerous form of NHA) cell lines. Both sera contained 10% v/v fetal bovine serum (~4 mg/mL total protein content). The NHA serum, in addition to the miscellaneous proteins from the fetal bovine serum, contained insulin and transferrin. The GBM serum contained no additional proteins.

Figure 7. AFM images of OTMS (left) and TESP (right) coated silicon wafers following incubation with NHA serum for 24h.

AFM images of the films exposed to the sera with 24 hours of incubation time under ambient conditions are shown in Figures 7 and 8 for NHA and GBM growth sera, respectively. In general, the OTMS surfaces appeared to be more heavily covered with proteinaceous material compared to the TESP coated surfaces. The rms roughness of the OTMS surface exposed to NHA growth serum was 11 Å, compared to 19 Å for the TESP surface. Although the TESP surface exposed to NHA growth media was rough by AFM, the adsorbed protein film appeared sparse

compared to the coverage on the OTMS surface. Surfaces exposed to GBM growth serum had rms roughness of 36 Å for the OTMS surface and 9 Å for the TESP surface. In the image of the OTMS coated wafer in Figure 8, there is a trough that appears to be mostly void of adsorbed protein. Such features in the protein-adsorbed surfaces were somewhat common. We believe that the cause originates from the rinsing procedure. That is, as the stream of rinse water pushes large, weakly adsorbed aggregates of proteins across the surface they, in turn, push away proteins in their path. The end result is micron scale troughs in the protein-adsorbed surface.
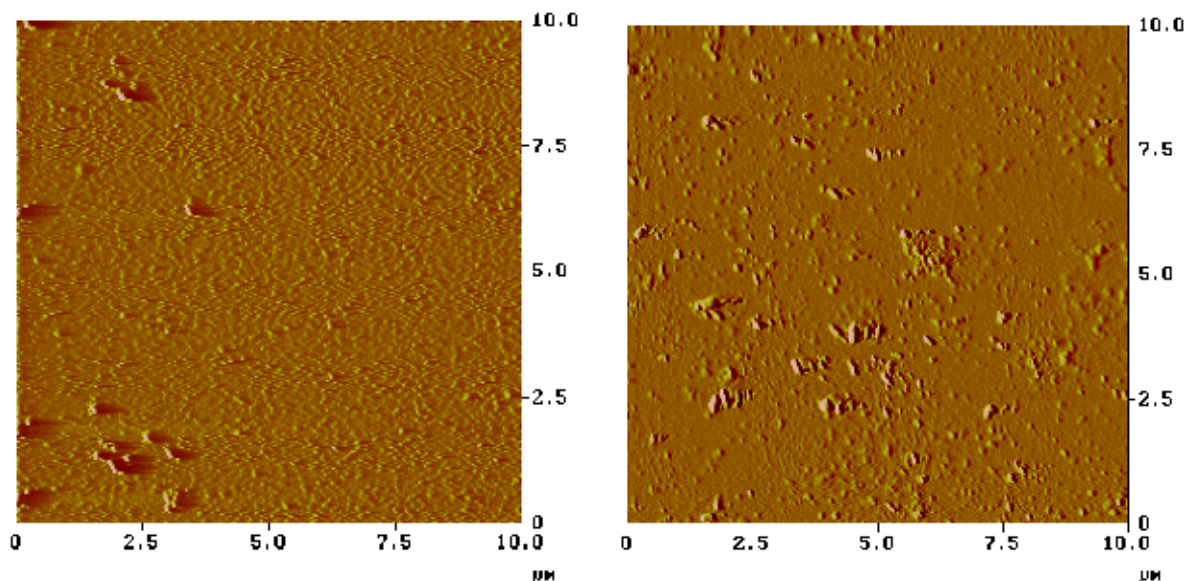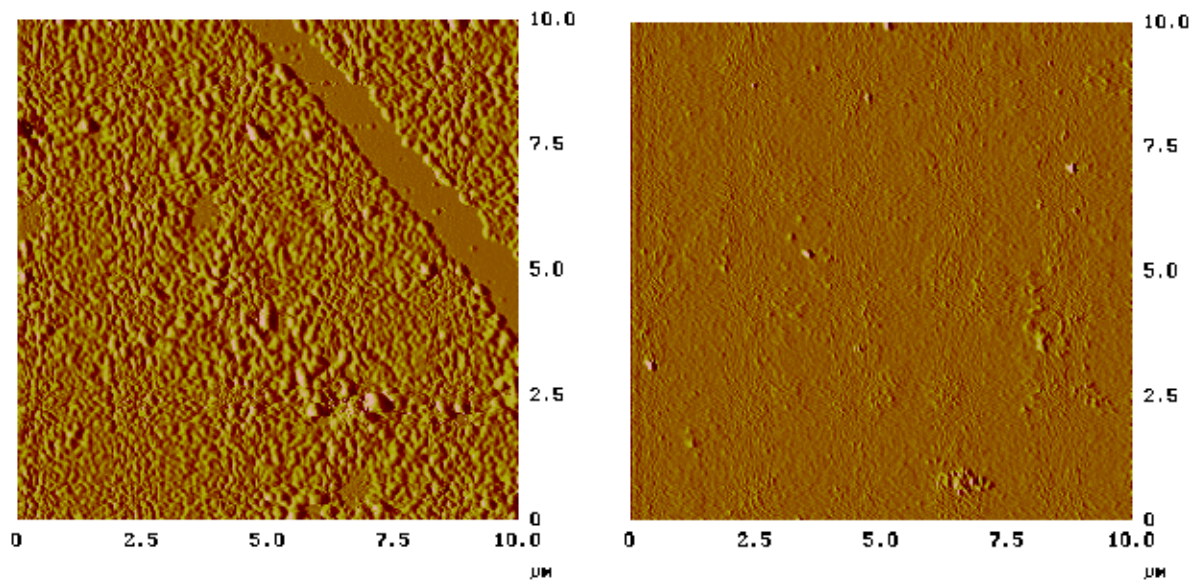

Figure 8. AFM images of OTMS (left) and TESP (right) coated silicon wafers following incubation with GBM serum for 24h.

Ellipsometry and water contact angle measurements generally agreed with the AFM results. Table 3 and Table 4 show the data for coated surfaces exposed to NHA and GBM growth sera, respectively. The data are not significantly different between the two sera for the hydrophobic and hydrophilic surfaces. With both sera, the hydrophobic OTMS surfaces adsorbed a protein film that was approximately twice as thick as that of the hydrophilic TESP coated surface after 2 hours. Similar to the BSA studies, the hydrophobic surface became relatively hydrophilic upon protein adsorption, while the hydrophilic surface became relatively hydrophobic. Some of the data in both Tables 3 and 4, such as the flip-flop of the film thickness and contact angles from 2 to 24 hours, were difficult to interpret. We are currently refining our experiments and data to determine the cause of these phenomena and further our understanding of protein adhesion on functionalized surfaces.

Table 3. Characterization of coated Si with exposure to NHA serum media

Coating	Water Contact Angle			Film Thickness (Å)	
	Initial	2h	24h	2h	24h
OTMS	105°	91°	76°	56 ± 5	36 ± 2
TESP	58°	82°	83°	24 ± 1	43 ± 2

Table 4. Characterization of coated Si with exposure to GBM serum media

Coating	Water Contact Angle			Film Thickness (Å)	
	Initial	2h	24h	2h	24h
OTMS	103	86	91	46 ± 1	64 ± 3
TESP	63	60	82	22 ± 1	0

Albumin is known to adsorb to both hydrophilic and hydrophobic surfaces but with very different modes of interaction (*23,24,27*). Albumin, adsorbed onto hydrophobic surfaces, rapidly denature and adhere strongly through van der Waals forces. Hydrophilic surfaces, in particular PEG coated surfaces, also adsorb protein but the protein maintains its native structure such that surface mobility and desorption is facile. When cells adhere to surfaces they coat their point of contact with vitronectin and fibrinogen, upon which the cells can anchor (*14,15,18*). For proteins weakly bound to the surface, such as BSA on PEG coated surfaces, replacement of BSA with fibrinogen readily occurs, promoting cell adhesion. On the other hand, the tenaciously bound BSA, denatured on the hydrophobic surface, is difficult for the cell to desorb and replace with fibrinogen resulting in poor cell adhesion. The protein aggregates on the TESP coated silicon and the flat, thick protein film on the hydrophobic OTMS coating are consistent with these aforementioned results. We will show in a later publication that cell adhesion in static and flow systems are influenced by protein adhesion on hydrophilic and hydrophobic coatings on semiconductor surfaces.

Conclusions

Surface passivation using SAM films on silicon effectively retards the formation of thick protein films. A hydrophilic SAM, prepared from a simple, commercially available PEO-functionalized silane, TESP, and a hydrophobic hydrocarbon OTMS SAM were readily and reproducibly formed as flat, smooth coatings on silicon surfaces that were stable in buffered aqueous solution. In the absence of any surface coating, a silicon wafer rapidly becomes coated with a 70 Å thick film of protein when exposed to solutions of BSA at cell growth sera concentrations. Protein adhesion on a silicon surface was reduced ~15% by using a hydrophobic OTMS SAM and by greater than 50% with the hydrophilic TESP coating. AFM images revealed that the protein adhesion on the OTMS surface occurred as a relatively smooth film, whereas on the TESP coating the proteins tended to adsorb as aggregates. It is conceivable that the denaturation of proteins on the hydrophobic surface would cause a flattening and smoothing of the adsorbed protein film. Proteins adsorbed on the PEO coated surface, however, may maintain their conformation and lead to aggregate formation. The BSA adsorption studies provided a model to evaluate the adhesion and film forming properties of proteins from complex mixtures used in cell growth sera. Studies with cell growth sera paralleled the BSA model studies and added further evidence to the effective surface passivation of hydrophilic TESP films to protein adsorption.

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