Nanoscale adhesion, friction and wear studies of biomolecules on silane polymer-coated silica and alumina-based surfaces

Bharat Bhushan^{1,*†}, Kwang Joo Kwak¹, Samit Gupta^{2,3,4}
and Stephen C. Lee^{2,3,4,†}

¹*Nanoprobe Laboratory for Bio- & Nanotechnology and Biomimetics,*

The Ohio State University, 201 West 19th Avenue, Columbus, OH 43210-1142, USA

²*Department of Biomedical Engineering, The Ohio State University, 473 West 12th Avenue, Columbus, OH 43210, USA*

³*Department of Chemical and Biomolecular Engineering, The Ohio State University, 473 West 12th Avenue, Columbus, OH 43210, USA*

⁴*Dorothy M. Davis Heart and Lung Research Institute, 473 West 12th Avenue, The Ohio State University, Columbus, OH 43210, USA*

Proteins on biomicroelectromechanical systems (BioMEMS) confer specific molecular functionalities. In planar FET sensors (field-effect transistors, a class of devices whose protein-sensing capabilities we demonstrated in physiological buffers), interfacial proteins are analyte receptors, determining sensor molecular recognition specificity. Receptors are bound to the FET through a polymeric interface, and gross disruption of interfaces that removes a large percentage of receptors or inactivates large fractions of them diminishes sensor sensitivity. Sensitivity is also determined by the distance between the bound analyte and the semiconductor. Consequently, differential properties of surface polymers are design parameters for FET sensors. We compare thickness, surface roughness, adhesion, friction and wear properties of silane polymer layers bound to oxides (SiO₂ and Al₂O₃, as on AlGaN HFETs). We compare those properties of the film–substrate pairs after an additional deposition of biotin and streptavidin. Adhesion between protein and device and interfacial friction properties affect FET reliability because these parameters affect wear resistance of interfaces to abrasive insult *in vivo*. Adhesion/friction determines the extent of stickage between the interface and tissue and interfacial resistance to mechanical damage. We document systematic, consistent differences in thickness and wear resistance of silane films that can be correlated with film chemistry and deposition procedures, providing guidance for rational interfacial design for planar AlGaN HFET sensors.

Keywords: adhesion; immunoFET; friction; silicon; AlGaN; heterojunction field-effect transistor sensors

1. INTRODUCTION

Protein layers are deployed on the surfaces of devices *in vivo* (such as biomicroelectromechanical systems, BioMEMS; Park 1997; Ozkan *et al.* 2003; Bhushan 2007) to confer molecular recognition (binding) properties (Lee *et al.* 2005; Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008) or enhance biocompatibility (Black 1999; Wise 2000). Multiple BioMEMS/NEMS protein sensors depend on proteins deployed on surfaces for their specific sensing properties, including field-effect transistor (FET) sensors (Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008), cantilever beam sensors (Lang *et al.* 2005; Bhalerao *et al.* 2007) and

quantum dot sensors exploiting Forster resonance energy transfer (FRET; Fan *et al.* 2005). When interfacial proteins are analyte receptors, loss of biomolecular function does not compromise the mechanical, electrical or quantum properties of the sensing transducers, but results in a loss of sensor biochemical specificity, causing sensor failure.

Protein sensor receptors can be damaged in multiple ways. Indwelling devices exhibit micromotion within tissue beds, producing proinflammatory tissue damage. This triggers the foreign body response (inflammatory infiltrates, formation of a fibrous capsule, etc.; Turbill *et al.* 1996), over days to weeks post-implantation, leading to mechanical and enzymatic damage of interfaces. Hydrolytic enzymes of inflammatory infiltrates damage interfacial proteins (proteolysis, protein chemical modification), and mechanical abrasion by

*Author for correspondence (bhushan.2@osu.edu).

†These authors contributed equally to the work.

Figure 1. Schematic of HFET-based bioFET sensor.

extracellular matrix (of the fibrous capsule) can cause receptor loss/damage and consequent sensor failure. Tissue abrasion associated with device deployment can also damage interfaces. Aside from the robustness of the sensing platform itself (FET, cantilever, etc.), the resistance of interfacial layers to insult is a design parameter that is often ignored.

Protein receptors for sensors are deployed on surfaces by direct adsorption or by conjugation to an interfacial polymer layer (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008), and proteins deposited by different methods have different wear properties. Deeper understanding of nanoscale adhesion, friction and wear of biomolecular interfaces supports rational changes in interfaces, improving sensor (and other BioMEMs) function *in vivo*, over time. Design modifications include selection of interfacial polymer layers with greater or lesser resistance to mechanical wear, greater or lesser thickness or porosity, strategies to improve resistance to wear (by enhancing cross-linking within interfacial layer(s), or to the micromachined substrate, or both) and protein engineering approaches that might affix receptors more stably and increase the resistance of individual proteins to damage.

We are developing planar AlGaN/GaN heterojunction field-effect transistor sensors (HFETs; figure 1; Shapiro *et al.* 2007; Gupta *et al.* 2008) for clinically important protein analytes. We (Shapiro *et al.* 2007; Gupta *et al.* 2008) and others (Kang *et al.* 2007) have shown that the classical dismissal of planar bioFETs for protein sensing in biological buffers/fluids (Bergveld 1991; Schoening & Poghossian 2002) is inappropriate. However, the classical assessment of bioFET feasibility is correct in asserting that the distances between analyte charges and semiconductor surfaces and buffer salt concentration are inversely related to signal intensity (Gupta *et al.* 2008).

To preserve receptor function and structure, receptors are conjugated via a polymeric layer covalently linked to the sensing surface, necessarily increasing the distance between the analyte charges and semiconductor. We used 3-aminopropyltriethoxysilane (APTES) on MOSFET interfaces, and have also used APTES in functional HFET protein sensors (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008). APTES layers are commonly described as self-assembled monolayers (SAMs), though this is often inaccurate (Kallury *et al.* 1994; figure 2). APTES interfaces are multilayered due to intermolecular polymerization, with significant

cross-linking between polymer monomers but sparse cross links between polymer and substrate. AFM data suggest that this is true of our interfaces (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Eteshola *et al.* 2008).

Interface thickness and robustness influence HFET sensitivity and stability, so with its propensity to form multilayers (Kallury *et al.* 1994; Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Eteshola *et al.* 2008), and its low mechanical strength (Bhushan *et al.* 2006a), APTES is a suboptimal interfacial material. Improved deposition protocols allow thinner, more regular, APTES interfaces to be made (Moon *et al.* 1996; Han *et al.* 2006), but multilayer formation remains an intrinsic potential of the reagent (Kallury *et al.* 1994). APTES monomers can form infinite, cross-linked siloxane polymer lattices because APTES is a multi(tri)-valent silane. Conversely, 3-aminopropyltrimethylethoxysilane (APDMES) cannot polymerize into extensive networks because it is monovalent, forming only siloxane dimers or linkages to substrate oxides (figure 2). APDMES should therefore produce thinner films of greater mechanical robustness than APTES, and HFET sensitivity and robustness should be improved by the thinner, more durable, interfaces.

Friction and wear properties of biomacromolecules are challenging to study. Macro-level protein lubrication effects on wear and friction properties of orthopaedic implants are documented (Liao *et al.* 1999; Scholes *et al.* 2000; Wang *et al.* 2004), but the relevancy of those studies to polymer-protein interfaces or to wear on much smaller BioMEMS/NEMS is uncertain, especially because tribological properties change between macro- and nanoscale regimes (Bhushan *et al.* 1995; Bhushan 1999, 2001, 2008). We studied the nanotribology of polymer-protein (streptavidin or STA) interfaces on micromachined SiO₂ (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Eteshola *et al.* 2008) to understand and optimize the performance of MOSFET protein sensors. We return to the nanotribology of these layers on microfabricated surfaces to compare the thickness and durability of APTES layers made with our original deposition procedure (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Eteshola *et al.* 2008), as well as an improved procedure (Han *et al.* 2006), and APDMES interfaces. We examine the thickness and durability of these polymer layers with receptor protein (streptavidin) bound to them. We compare the nanotribology of these interfaces on SiO₂ and Al₂O₃ surfaces (Al₂O₃ is the most common oxide on AlGaN surfaces, and so it is used as a model for the much more expensive AlGaN substrate) for rational redesign of HFET sensor interfaces.

2. EXPERIMENTAL

2.1. Reagents

We use siloxane polymers to construct polymer layers to which analyte receptor proteins are affixed in our FET protein sensors (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008). 3-APTES and 3-APDMES were purchased from Gelest (Morrisville, PA) and were used to construct polymer layers on metal

Figure 2. Schematics of (a) idealized APTES, (b) multilayered APTES and (c) idealized APDMES silane polymer films on silicon/ SiO_2 and aluminium/ Al_2O_3 substrates. Idealized film corresponds to a self-assembled monolayer.

oxide substrates. Fluorescently labelled streptavidin (streptavidin–Alexa Fluor 488) was purchased from Pierce Biotechnology (Rockford, IL). Sulpho-*N*-hydroxysuccinimidobiotin (sulpho-NHS-biotin) was also purchased from Pierce and used to link streptavidin–Alexa Fluor 488 to the silane polymer layers.

2.2. Substrates

Two substrates, silicon with a SiO_2 oxide layer (silicon/ SiO_2) and aluminium with an Al_2O_3 oxide layer ($\text{Al}/\text{Al}_2\text{O}_3$), were used in this study. Silicon/ SiO_2 is a common micromachined surface, and $\text{Al}/\text{Al}_2\text{O}_3$ is a model for oxidized AlGaN surfaces (see below).

2.2.1. Silicon/ SiO_2 substrate cleaning. Semiconductor-grade silicon wafers (identical to those used in Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005; Eteshola *et al.* 2008) were purchased from Wafer World, Inc. (West Palm Beach, FL). Diced silicon wafers were prepared as described previously (Lee *et al.* 2005), with one modification. Instead of an HF etch prior to surface treatment, the wafers were treated in piranha solution (3 : 1 H_2SO_4 : H_2O_2) for 30 min at room temperature and then rinsed with DI water. Silicon wafer surfaces were hydroxylated by boiling for 30 min in DI water or by a 30 min treatment of HCl:methanol (1 : 1 by volume; Han *et al.* 2006).

2.2.2. Aluminium/ Al_2O_3 substrate. A 100 nm thick Al layer was sputter coated on a cleaned silicon wafer in a high-vacuum chamber at a pressure of 5×10^{-7} torr. After sputtering, an alumina (Al_2O_3) layer formed spontaneously at the surface from oxidation in air. Al-coated wafers were hand diced as described for cleaned

silicon wafers (Lee *et al.* 2005). Al-coated wafer surfaces were hydroxylated by boiling for 30 min in DI water.

2.2.3. Deposition of silane polymer layers. Siloxane polymers were deposited on the substrates prepared as above. Two different methods were used for deposition of APTES on the surfaces. For the APTES (1) protocol, silicon wafers and Al-coated ones were hydroxylated by boiling. Following hydroxylation, samples were incubated for 30 min in 2 per cent (by volume) APTES in acetone at room temperature. The samples were then rinsed three times with acetone and N_2 dried. The APTES (2) protocol is derived from that of Han *et al.* (2006) who report their protocol to eliminate deposited multilayers of APTES, leaving primarily APTES molecules that are in contact with substrate oxides. For the APTES (2) protocol, samples were hydroxylated by boiling (Al-coated substrates) or by HCl:methanol (1 : 1 by volume) for 30 min treatment (silicon substrates). The samples were then immersed for 12–15 hours in a 5 per cent (v/v) APTES solution in ethanol at 50°C . Then the samples were rinsed with 1 M acetic acid and rinsed twice with absolute ethanol to eliminate excess APTES. The protocol for deposition of APDMES was the same as that for APTES (2) on each respective substrate (Al-coated and silicon substrates), with the exception that the APDMES precursor was used.

2.2.4. Biotinylation of silane polymer layers and deposition of streptavidin. We used sulpho-NHS-biotin dissolved in DI water (1 mg ml^{-1}) to covalently append biotin to siloxane layers. This reaction, as well as subsequent attachment of streptavidin to the biotinylated interface, takes place as described previously (Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005).

2.3. Surface measurements and tribology of films on substrates

2.3.1. Thickness measurements. The film thickness was measured using an ellipsometer with a nominal thickness resolution of 0.2 nm (Gaertner Scientific LI15CLC waferscan with LI16 microspot optics). A He–Ne laser with a beam wavelength of 632.8 nm was used, with the angles of reflection and incidence set at 70°. The polarizer drum was fixed at 45°. The laser beam projected on the sample surface produced an elliptical spot size of approximately $30 \times 50 \mu\text{m}^2$. The uncoated wafer was mounted on the sample stage. By looking through the eyepiece and rotating the X- and Y-plane tilt adjustment controls, sample surface out-of-flatness conditions were corrected. The real (N_s) and extinction values (K_s) of the refractive index of the sample, which is Si with a very thin film of native oxide, were measured; typical measured values for N_s and K_s were 4.00 and 0.30, respectively. For comparison, the refractive index of Si is 3.49 (Lide 2003), and the value for SiO_2 is 1.46 (Shackelford & Alexander 2001). Five measurements at various locations were made by translating the Si-based sample with the aid of a stepper motor. The N_s and K_s (measured previously) and the refractive indices of APTES and APDMES, which are 1.4225 and 1.4276, respectively (Gelest Silanes and Silicones Catalog 4000A, 2008), were entered into the film measurement subprogram in the software. The film thickness was measured using the algorithm provided by the manufacturer.

2.3.2. Static contact angle measurements. Droplets of approximately 5 μl in volume (with a radius of a spherical droplet of approx. 1 mm) were gently deposited on the substrate using a microsyringe for the static contact angle. The image of the droplet was obtained by a digital camcorder (DCR-SR100; Sony, Tokyo, Japan) with a 10 $\times$ optical and 120 $\times$ digital zoom. Images obtained were analysed using IMAGETOOL software (University of Texas Health Science Center) for the contact angle.

2.3.3. Surface morphology, adhesion, friction and wear. Surface morphology, adhesion, friction and wear measurements were made using an AFM (Digital Instruments, Santa Barbara, CA) in air and a liquid environment. In air AFM, a commercial cantilever holder was used in the contact mode. Square pyramidal etched single-crystal Si probes (RFESP; Digital Instruments, Santa Barbara, CA) with a radius of 10 nm on a rectangular beam with a spring constant of 3 N m^{-1} were used. In the general operation of a commercial liquid AFM, the whole liquid cell is excited by a piezoelectric element, which results in a multitude of spurious peaks related to the fluid cell eigenfrequencies (Bhushan *et al.* 2005, 2006a). In this paper, a modified cantilever holder (Maali *et al.* 2006; figure 3) was used to directly provide piezo excitation to the cantilever in fluid with a commercial MultiMode III AFM in the tapping mode. A horizontal slot was carved out in the opening of the commercially available cantilever holder for non-fluid use above the piezo element in order to

Figure 3. Schematic of a modified cantilever holder used to directly provide piezo excitation to the cantilever in fluid with a commercial MultiMode III AFM in the tapping mode (Maali *et al.* 2006).

insert a glass slide. When the liquid is added between the glass slide and the substrate, the liquid meniscus is formed between the glass and the sample surface for fluid imaging. The working principle of a liquid cell is similar to that for the normal tapping mode. For these studies, square pyramidal Si_3N_4 probes (OTR4-TR; Digital Instruments, Santa Barbara, CA) with a nominal 15–20 nm tip radius mounted on triangular Si_3N_4 cantilevers with a spring constant of 0.08 N m^{-1} and a resonance frequency of 8–9 kHz were used.

The adhesion measurements were made in force calibration mode using a Si or Si_3N_4 AFM tip in air and water, respectively (Bhushan 1999, 2007, 2008). The adhesive force for each force calibration plot was obtained by multiplying the maximum deflection of the cantilever in the retract position with the spring constant of the cantilever. Adhesion measurements were carried out by a Si AFM tip against silica and alumina surfaces coated with silane polymer films in air. For the liquid environments, a Si_3N_4 AFM tip was used against silica and alumina surfaces coated with silane polymer films in deionized (DI) water and with biomolecules over silane polymer films in a PBS solution. Multiple measurements were made for statistical analysis of the data.

Friction tests were conducted using an AFM in the contact mode, referred to as friction force microscopy (FFM). The experimental procedure in this study was based on Ruan & Bhushan (1994; see also Bhushan 1999, 2007, 2008.) In this method, the sample is scanned perpendicular to the long axis of the cantilever beam and the output of the two horizontal quadrants of the photodiode detector is measured. In this arrangement, as the sample moves under the tip, the friction force will cause the cantilever to twist. Therefore, the light intensity between the left and right detectors will be different. The differential signal between the left and right detectors is denoted as FFM signal. This signal can be related to the degree of twisting and hence to the magnitude of friction force. Owing to a possible error in determining normal force due to the presence of an adhesive force at the tip–sample interface, the slope of the friction data (FFM signal versus normal load) must be taken for an accurate measurement of the coefficient of friction. The tests were repeated at least three times to determine the average value of the coefficient of friction. Multiple experiments for each test matrix were conducted to verify the trends.

Wear tests were conducted in the contact mode in air and in the tapping mode in liquid environments. For wear tests of silane polymer films in air, a $1 \times 1 \mu\text{m}^2$ area was scanned at a normal load of 100 nN and then a $3 \times 3 \mu\text{m}^2$ area was imaged and the average wear depth was calculated. For wear tests in PBS, a $1 \times 1 \mu\text{m}^2$ area was scanned at a desired normal load and then a $3 \times 3 \mu\text{m}^2$ area was imaged at minimum possible normal load, to image the wear marks without any distortion. The excitation frequency in the tapping mode used was 8.5 kHz. By adjusting the drive amplitude voltage (voltage supplied to the piezoelectric vibrator), the initial amplitude setpoint was maintained at approximately 1.0 V. The approximate applied normal load in the tapping mode can be obtained by multiplying the amplitude setpoint voltage (V), the conversion factor (50 nm V^{-1}) obtained from the AFM settings and the cantilever stiffness (0.08 nN nm^{-1}). The equivalent calculated normal load for 50 per cent of the free tapping amplitude is 2 nN. Multiple experiments for each test matrix were conducted to verify the trends.

After wear tests in air, the friction force images were obtained to corroborate the wear data. Friction force images in PBS did not provide reproducible data. Instead, phase angle images were obtained in the tapping mode. The feedback signal in the Z-direction sample piezo (to keep the setpoint constant) is a measure of surface height. The extender electronics of the instrument were used to measure the phase angle lag between the piezoelectric vibrator drive signal and the cantilever response during engagement (Bhushan & Qi 2003; Scott & Bhushan 2003).

3. RESULTS AND DISCUSSION

3.1. Characterization, adhesion and friction of films

3.1.1. Films on silicon/ SiO_2 . The potential 'height' (distance from the polymer amine group to the substrate) of APTES and APDMES monolayers (assuming one achieves them) is identical and can be approximated by summing bond lengths of each compound (approx. 1.2 nm; Shapiro *et al.* 2007). Based on the silane literature (Kallury *et al.* 1994; Han *et al.* 2006), and on the specific siloxane chemistry of the silane monomers APTES (trivalent, capable of forming extensive intramolecular polymers) and APDMES (monovalent, capable of intramolecular dimerization), a rank order of silane layer thicknesses can be hypothesized. From the thickest to thinnest, the expected order is (i) APTES deposited by APTES (1) protocol, (ii) APTES deposited by APTES (2), and (iii) APDMES (deposited here by the APTES (2) method).

Figure 4 shows ellipsometry measurement of each of these films, and corroborates the predicted rank order of thickness. Furthermore, the result for APDMES is very similar to the length of the molecule calculated from bond lengths. Both APTES layers are considerably thicker, suggesting the formation of multilayers. We previously came to a similar conclusion regarding the multilayered nature of APTES films deposited by APTES (1) based on the AFM data (Bhushan *et al.*

2005; Lee *et al.* 2005), and both APTES (1) and (2) seem to result in multilayered APTES layers. However, APTES (2)-deposited APTES films may be marginally thinner than APTES (1)-deposited APTES films. Contact angle measurements show that as films more closely approximate genuine SAMs hydrophobicity increases (Bhushan & Liu 2001; Liu & Bhushan 2002; Bhushan *et al.* 2006b), congruent with our earlier comparisons of contact angle of silicon substrates and APTES films (Bhushan *et al.* 2005; Lee *et al.* 2005). The APDMES film approximates predicted monolayer thickness, but since we cannot certify that every substrate oxide group is derivatized with an APDMES monomer and that there are no 'bare' (uncovered) sites on the substrate, we hesitate to declare that film a perfect SAM.

Also consistent with the apparent differential extents of multilayering of different APTES and APDMES layers, the rank order of roughness and maximal peak–valley (P–V) distances measured by AFM follow the same rank order as thickness measured by ellipsometry, with APTES deposited by the APTES (1) procedure being most rough, with the greatest P–V values, and APDMES being the smoothest, with the least P–V values (figure 4). Similar trends manifest for the three films in air. Notably, P–V values for the substrate and APDMES film are virtually identical, suggesting that the APDMES film height profile is determined by the height profile of the substrate (table 1), while that of the APTES films (particularly, the film deposited by APTES (1) methods) is substantially greater (approx. twofold) than that of the substrate. This again suggests that the APDMES film may approximate a genuine SAM, while both APTES films do not.

As expected, the adhesive force is greater in air than water, presumably due to meniscus contribution (Bhushan 1999, 2008). Rank order of adhesive force is inversely related to the contact angle, with APDMES films exhibiting the lowest adhesive force (Bhushan 1999, 2008). On the other hand, coefficients of friction do not exhibit the APTES (1) to APDMES rank order, perhaps reflecting differential shear strength of the three films.

The next layer of interfaces used in our sensors (Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008) is provided by the reaction of sulpho-NHS-biotin with the silane polymer layer. Accordingly, each of the interfacial polymer layers used in figure 1 were reacted with sulpho-NHS-biotin and evaluated by AFM (figure 5; table 1). Treatment of the silane layers with sulpho-NHS-biotin increases the thickness of the films (Bhushan *et al.* 2005; Lee *et al.* 2005; Shapiro *et al.* 2007). The roughest film (APTES deposited by APTES (1); figure 4; table 1) remains by far the roughest after sulpho-NHS-biotin deposition, with the largest P–V value (table 1). Interestingly, appending sulpho-NHS-biotin to APTES deposited by APTES (2) apparently smooths the interface relative to APTES alone (table 1). For that matter, sulpho-NHS-biotin on APDMES is marginally smoother than is APDMES alone. We speculate that, because the terminal group of sulpho-NHS-biotin (i.e. biotin) is considerably larger

Figure 4. Representation of (a) film thickness, (b) surface roughness (RMS and P–V distance), (c) contact angle, (d) adhesive force (in air and DI water) and (e) coefficient of friction (in air and DI water) for various silane polymer films as well as SiO₂ substrate (for reference). The error bars represent $\pm 1\sigma$.

than the terminal amine of either APTES or APDMES, it may be able to fill in the imperfections in films that, like APDMES, and perhaps APTES deposited by APTES (2), have relatively regular orientations of the silane polymer and are more or less uniformly deposited on the substrate (see table 1 for roughness and P–V values of the respective films in air; figure 2). We further speculate that because of the orientation of APTES amine groups (and therefore the orientation of biotin end groups after conjugation with sulpho-NHS-biotin), the APTES (1) APTES are considerably less regular and APTES (1) APTES films are rougher than the other films (figures 2 and 4; table 1), appending sulpho-NHS-biotin may not be able to homogenize the apparent film surface height of the APTES film deposited by the APTES (1) protocol.

We use streptavidin as a receptor in our validation of planar HFET protein sensing (Shapiro *et al.* 2007; Eteshola *et al.* 2008; Gupta *et al.* 2008), and so here

we compare the interfaces after biotinylation with sulpho-NHS-biotin and streptavidin binding. Roughness and P–V values follow the trends observed upon appending sulpho-NHS-biotin to silane layers observed above, with the APDMES-based interface having the lowest roughness and P–V values (figure 5; table 1), though the differential roughness of the three interfaces is less pronounced after binding of streptavidin. Streptavidin is a large space-filling structure (approx. 8 nm diameter), and the reduced differential roughness between the three interfaces may reflect streptavidin's packing properties at high densities, as at these interfaces.

Lastly, for all siloxane interfaces, adhesion and coefficients of friction are the smallest for interfaces after streptavidin binding. This is consistent with previous reports of the lubricating properties of proteins (Liao *et al.* 1999; Scholes *et al.* 2000; Wang *et al.* 2004; Bhushan *et al.* 2006a), and may reflect the

Table 1. Roughness values of sample surface in air and PBS buffer environment using an AFM ($3 \times 3 \mu\text{m}^2$).

specimen	silane polymer (air)		biotin over silane polymer (PBS)		STA-biotin over silane polymer (PBS)	
	RMS ^a (nm)	P–V ^b (nm)	RMS (nm)	P–V (nm)	RMS (nm)	P–V (nm)
SiO ₂ substrate	0.9	7				
APTES (1)	1.4	14	1.5	17	1.4	15
APTES (2)	1.3	12	0.8	13	0.9	14
APDMES	1.0	11	0.7	11	0.8	13
Al ₂ O ₃ substrate	2.8	32				
APTES (1)	3.3	42	4.6	42	3.7	38
APTES (2)	2.6	23	3.6	48	4.1	47
APDMES	2.8	24	4.5	54	3.7	53

^aRMS, root mean square roughness.^bP–V, peak-to-valley distance.

Figure 5. Representations of surface properties of indicated silane films deposited on silicon/SiO₂. (a) Roughness (RMS, P–V distance) of silane films with biotin appended. (b) Roughness of biotinylated films with streptavidin (STA) bound to them. (c) Adhesive force measurements of indicated biotinylated silane films and the same biotinylated films with STA bound to them. (d) Coefficients of friction for indicated biotinylated silane films and the same biotinylated films with STA bound to them. The error bars represent $\pm 1\sigma$.

fact that after streptavidin is bound to these interfacial polymer layers, the properties of streptavidin dominate the adhesion and friction properties of the surfaces rather than those of the underlying polymers. This is intuitively pleasing as streptavidin is densely bound to biotinylated polymer layers (Lee *et al.* 2005; Bhushan *et al.* 2005, 2006a,b; Shapiro *et al.* 2007) and the size of the streptavidin tetramer (8 nm) is much greater than the size of any of the individual polymer monomers (less than 2 nm each for silanes and sulpho-NHS-biotin).

3.1.2. Films on aluminium/Al₂O₃. Our functional planar protein sensors are AlGa_N heterojunction FET (HFET) devices (Shapiro *et al.* 2007; Gupta *et al.* 2008), on which we have deposited APTES siloxane layers using the APTES (1) protocol followed by

sulpho-NHS-biotin and streptavidin. Oxides on the AlGa_N surface are known to include both aluminium and gallium compounds, but the most prevalent oxide is Al₂O₃ (discussed in Gupta *et al.* 2008). From the standpoint of sensor function, we have been able to deposit acceptable APTES films on AlGa_N devices (Shapiro *et al.* 2007; Gupta *et al.* 2008), and good quality polymer films (thin, smooth and regular with good adhesion and roughness properties) on Al₂O₃ have been demonstrated with non-silane polymer chemistries (phosphonate chemistries; Hoque *et al.* 2006), so good quality silane films on AlGa_N may also be possible. We therefore wished to determine whether some of the characteristics of our interfaces on AlGa_N parallel those we observed on silicon wafers, but were stymied because epitaxially grown AlGa_N surfaces are

Figure 6. Representations of surface properties of indicated silane films deposited on aluminium/ Al_2O_3 . (a) (i) Thickness of the indicated films as determined by ellipsometry and (ii) comparison of the roughness of the indicated films (RMS, P-V) to that of the aluminium/ Al_2O_3 substrate. (b) (i) Roughness (RMS, P-V) of the indicated films with biotin appended to them, (ii) roughness (RMS, P-V) of the indicated biotinylated films with STA bound to them, (iii) adhesive force measurements of indicated biotinylated silane films and the same biotinylated films with STA bound to them and (iv) coefficients of friction for indicated biotinylated silane films and the same biotinylated films with STA bound to them. The error bars represent $\pm 1\sigma$.

extremely expensive. It is inexpensive to deposit an aluminium layer on silicon wafers by sputter coating, allow it to spontaneously form an Al_2O_3 oxide on contact with air and then deposit interfaces by the methods discussed above, to model the expected properties of interfaces on AlGaIn. This approach is limited, in that silicon and AlGaIn wafers have extremely smooth surfaces (table 1; Bhushan *et al.* 2005, 2006a; Lee *et al.* 2005), while sputter-coated aluminium surfaces are much rougher (table 1), but some inferences can be drawn.

Ellipsometry data (figure 6) show that the thinnest film on sputter-coated aluminium with an oxide layer is APDMES, with a thickness similar to that expected for an APDMES monolayer. Presumably, this would also be the case on AlGaIn surfaces. Figure 6 shows that P-V values for both APTES (APTES (2)) and APDMES are considerably lower than those for APTES (APTES (1)),

and to this extent are consistent with the observation of the same polymer layers on silicon substrates. These data suggest that silane layers largely follow the contours of the aluminium/ Al_2O_3 substrate. That said, once biotin and streptavidin are appended to the polymeric surfaces, previously observed trends in roughness and P-V values on silicon/ SiO_2 are not well reproduced: APDMES-based films may be no smoother than those based on APTES deposited by APTES (2). APDMES on aluminium/ Al_2O_3 does not have the lowest roughness, though it did on the SiO_2 substrate. Disparities between the silicon/ SiO_2 and aluminium/ Al_2O_3 substrates may follow from the fact that the Al_2O_3 substrate is three times rougher than the SiO_2 substrate and is much less uniform (table 1). There may also be differences in the density and distribution of APDMES monomers in APDMES films on (rough) Al_2O_3 surfaces. That is, sputter-coated aluminium may

not be a perfect model of epitaxially grown AlGaN, but it does show us that, of the silane films tested, APDMES makes the thinnest layers on Al_2O_3 surfaces, and should therefore produce the thinnest films on AlGaN. Ultimately, the significance to HFET protein sensors of other disparities observed between interfaces on silicon/ SiO_2 and aluminium/ Al_2O_3 will be resolved by comparison of interfaces deposited on silicon and AlGaN surfaces.

3.2. Wear of films

3.2.1. Films on silicon/ SiO_2 . Figure 7 shows AFM surface height and friction force images and cross-sectional profiles obtained after wear test in air in the contact mode at 100 nN on bare SiO_2 , APTES (1), APTES (2) and APDMES samples. The data demonstrate differential wear of the two APTES and APDMES surfaces in silicon/ SiO_2 . At identical loads, the APTES (1) film exhibits the greatest wear, followed by the APTES (2) film, and then the APDMES film. The hierarchy in wear reflects previously demonstrated polymer layer-specific trends in thickness, roughness, adhesion and friction coefficients. Friction force data follow the same rank order and is indicative of relative physical wear. The data suggest that, of the three films, APDMES is the most well organized and has the highest wear resistance.

The wear experiments in PBS buffer were carried out in the tapping mode in order to perform tests under gentler conditions. As controls, we examined the wear of silicon/ SiO_2 surfaces with no silane polymer layer that had been treated with NHS biotin (figure 8a(i)) and then further treated with streptavidin (figure 8a(ii)). In these controls biotin and streptavidin can adhere to the surface only by non-specific adsorption, and are easily removed from the silicon/ SiO_2 surface, much as we have shown in the past (Bhushan et al. 2006a). Figure 8 shows that the trend in wear observed in air is conserved in the same interfaces further derivatized with sulfo-NHS-biotin and streptavidin, and under PBS buffer. The rank order of film resistance to wear is as expected: more highly multilayered APTES films (represented by those deposited using the APTES (1) protocol) are expected to have higher levels of cross-linking within the film itself but lower densities of APTES to substrate oxide bonds (Kallury et al. 1994; Han et al. 2006). The APTES (2) protocol removes excess multilayers of APTES (Han et al. 2006), increasing the total percentage of siloxane bonds occurring between APTES and the substrate, with the expected result of increasing the resistance of the APTES (2) interface to mechanical insult. Given that each molecule of APDMES must be bonded to a surface oxide group and that intrapolymer cross links are not possible in APDMES, it has the highest density of siloxane linkages to the substrate oxide, making it theoretically the most robust of the three interface layers. The expected higher density of APDMES cross links to the substrate oxide follows *a priori* from the relative valencies of APTES and APDMES, particularly as it relates to the inability of APDMES to form infinite lattices of its monomers, and is also congruent

Figure 7. AFM surface height and friction force images and cross-sectional profiles obtained after wear test in air in the contact mode at 100 nN loads for silicon/ SiO_2 : (a) unmodified substrate, (b) APTES deposited using the APTES (1) protocol, (c) APTES deposited using the APTES (2) protocol and (d) APDMES deposited using the APTES (2) protocol. Locations of cross-sectional profiles are indicated by the white lines.

with the higher robustness of APDMES films (see below). The hypothesized greater density of cross links to substrate might be confirmed spectrophotometrically (FT-IR, XPS), though the fact remains that APDMES interfaces are the most wear-resistant polymer films tested.

Additionally, since APDMES cannot efficiently form cross links between individual polymer monomers, force applied by the cantilever cannot be transferred from one APDMES molecule to another throughout a cross-linked polymer sheet. Such diffusion of force applied to

Figure 8. Differential wear of interfacial layers on silicon/SiO₂ worn in PBS in tapping mode at 50% free amplitude (approximately 2 nN load.) (a) (i) No silane polymer layer but exposed to NHS biotin as described for biotinylation of silane polymer films and (ii) no silane polymer layer but exposed to NHS biotin as in (a) (i) but further exposed to STA, as described in the text. (b) (i) APTES deposited by the APTES (1) protocol and biotinylated as described, (ii) APTES deposited by the APTES (1) protocol, biotinylated as described and with STA bound, (iii) APTES deposited by the APTES (2) protocol and biotinylated as described, (iv) APTES deposited by the APTES (2) protocol, biotinylated as described and with STA bound, (v) APDMES deposited by the APTES (2) protocol and biotinylated as described and (vi) APDMES deposited by the APTES (2) protocol, biotinylated as described and with STA bound. Locations of cross-sectional profiles are indicated by the white lines.

the interface might increase the robustness of APTES films until rupture force is exceeded on individual siloxane bonds to the substrate, but once that force is achieved, further application of force would be expected to tear away an entire section of cross-linked APTES sheet. In this aspect, cross-linked APTES layers may be somewhat analogous to graphene layers of graphite. Interaction across (within) the APTES sheet is prevalent and therefore the sheet itself is strong, but interaction between the sheet and its underlayer (the substrate) is sparse, and therefore attachment of the APTES sheet to the surface is relatively weak. By

contrast, force applied to one APDMES molecule would be expected to pluck that APDMES molecule from the surface and not be diffused by transfer to neighbouring, cross-linked molecules. We observe that the robustness properties of the various siloxane films on silicon/SiO₂ seem to determine the relative robustness of interfaces to which sulfo-NHS-biotin and streptavidin have been appended. However, AFM surface height and phase angle images and cross-sectional profiles of biotin and streptavidin-biotin over silane polymer films obtained after wear in PBS in the tapping mode at 50 per cent of free amplitude (higher loads, approx. 2 nN) indicate

Figure 9. AFM surface height and friction force images and cross-sectional profiles obtained after wear test in air in the contact mode at 100 nN loads for aluminium/ Al_2O_3 : (a) unmodified substrate, (b) APTES deposited using the APTES (1) protocol, (c) APTES deposited using the APTES (2) protocol and (d) APDMES deposited using the APTES (2) protocol. Locations of cross-sectional profiles are indicated by the white lines.

the applied force to be sufficient to debride interface constituents from the surfaces with all three polymer layers, so all three interfaces with streptavidin on silicon/ SiO_2 surfaces are delicate.

3.2.2. Films on aluminium/ Al_2O_3 . Figure 9 shows AFM surface height and friction force images and cross-sectional profiles obtained after wear test in air in the contact mode at 100 nN on bare aluminium/ Al_2O_3 , APTES (1), APTES (2) and APDMES samples. APDMES exhibits the lowest wear, just as it did on

silicon/ SiO_2 substrates. AFM surface height and phase angle images and cross-sectional profiles of silane layers biotinylated with sulpho-NHS-biotin and biotinylated silane layers with streptavidin bound to them after wear in PBS in the tapping mode at 50 per cent of free amplitude (approx. 2 nN) are shown in figure 10. NHS biotin (figure 10a(i)) and streptavidin were deposited on aluminium/ Al_2O_3 by direct adsorption, just as they were on silicon/ SiO_2 in figure 8 and were easily worn off the aluminium/ Al_2O_3 surface, much as they were easily worn off the silicon/ SiO_2 surface. Wear trends are the same as those for samples with silicon/ SiO_2 substrates. Despite large differences in the roughness of aluminium/ Al_2O_3 substrates relative to silicon/ SiO_2 substrates, and despite disparities in roughness adhesion and friction properties of the films on the two substrates (figure 6), conservation of wear trends from silicon/ SiO_2 to aluminium/ Al_2O_3 encourages us to speculate that rank order of durability results from films on silicon/ SiO_2 may be directly translatable to similar films on the bona fide AlGaN surfaces.

3.3. Wear of films as a function of load

We have demonstrated a rank order in the wear properties of various silane films and their biotinylated and streptavidin-bound derivatives on both silicon/ SiO_2 and aluminium/ Al_2O_3 substrates. For any of the films tested, on either of the substrates, one would expect wear to be directly related to load. We compared wear as a function of load for our thinnest, most robust film (APDMES), its biotinylated and streptavidin-treated derivatives, deposited on silicon/ SiO_2 (figure 11).

AFM surface height and friction force/phase angle images and cross-sectional profiles obtained after wear test in PBS in the contact/tapping mode at a range of normal loads/free amplitude are shown in figure 11a. The data for APDMES show that there is a critical load above which the silane polymer layer wears rapidly. This observation is consistent with the wear mechanisms reported by Bhushan & Liu (2001), Liu & Bhushan (2002) and Bhushan *et al.* (2006b) for various SAMs. For the biological films (APDMES biotinylated with sulpho-NHS-biotin and APDMES biotinylated with sulpho-NHS-biotin and with streptavidin bound), wear increases with applied normal load, as indicated by both surface height and phase images. At any given load, surfaces with streptavidin bound to them exhibit the greatest (deepest) wear because the sulpho-NHS-biotin and streptavidin layers provide a cushion leading to tip penetration into the film as reported by Bhushan *et al.* (2006a).

The average wear depth and average friction force/phase angle change as a function of average normal load is plotted in figure 11b. The relationship between the average wear depth and the average normal load is linear, though consistent with the depth profile data, the slope of the wear/depth to load is the steepest for the interface to which streptavidin has been bound. The average wear depths for APDMES after wear tests at 100, 200 and 300 nN loads are 0.2, 0.3 and 1.3 nm, respectively. At average normal load less

Figure 10. Differential wear of interfacial layers on aluminium/ Al_2O_3 worn in PBS in tapping mode at 50% free amplitude (approximately 2 nN load). (a) (i) No silane polymer layer but exposed to NHS biotin as described for biotinylation of silane polymer films and (ii) no silane polymer layer but exposed to NHS biotin as in (a) (i) but further exposed to STA, as described in the text. (b) (i) APTES deposited by the APTES (1) protocol and biotinylated as described, (ii) APTES deposited by the APTES (1) protocol, biotinylated as described and with STA bound, (iii) APTES deposited by the APTES (2) protocol and biotinylated as described, (iv) APTES deposited by the APTES (2) protocol, biotinylated as described and with STA bound, (v) APTES (2) protocol and biotinylated as described and (vi) APDMES deposited by the APTES (2) protocol, biotinylated as described and with STA bound. Locations of cross-sectional profiles are indicated by the white lines.

than 200 nN, the acceptable levels of wear are shown for APDMES layer on SiO_2 substrate. The average friction force values are 0.1, 0.2 and 0.6 nN, respectively. Average wear depths for streptavidin on sulpho-NHS-biotin for 50, 30 and 10 per cent of the free tapping amplitude (approx. 2, 3 and 4 nN average normal loads, respectively) are 0.7, 1.7 and 2.7 nm, respectively. Average phase angle changes for 50, 30 and 10 per cent of the free amplitudes for STA-biotin are 0.14° , 0.42° and 0.95° , respectively. The wear depth at 10 per cent of the free amplitude is considerable and is comparable to that of the wear in the contact mode.

The results suggest that the wear depth of film increases linearly with an increase in the normal load.

4. CONCLUSIONS

We have shown that there is a consistent rank order of thickness, roughness and susceptibility to mechanical wear for APDMES and APTES silane films on silicon/ SiO_2 substrates that can be rationalized by their known chemistries and expected deposition properties. APDMES is the most wear resistant, thinnest and smoothest. These parameters of APTES

Figure 11. Differential wear of derivatized APDMES films deposited on silicon/SiO₂ by the APTES (2) protocol at indicated loads and free amplitudes documented via AFM surface height and friction force/phase angle images. (a) (i) APDMES films worn in air, (ii) APDMES films with biotin appended to them worn under PBS in tapping mode and (iii) surface biotinylated APDMES films with STA bound to them worn under PBS in tapping mode. Locations of cross-sectional profiles are indicated by the white lines. (b) Graphical depiction of data from (a) (i–iii).

films vary with the deposition protocol, with films made with deposition methods reported to result in minimization of multilayer formation to be smoother, thinner and stronger. These trends are replicated when the silane layer is biotinylated and when streptavidin is bound to the biotinylated silane. Furthermore, wear and thickness trends are largely replicated with similar films deposited on aluminium/ Al_2O_3 substrates. Though roughness, coefficients of friction, adhesion and P–V values do not perfectly reproduce the trends observed with similar interfaces on silicon/ SiO_2 , the data from aluminium/ Al_2O_3 substrates suggest that the trends in robustness and thickness parameters seen on silicon/ SiO_2 will translate to AlGaIn surfaces, suggesting that APDMES may be a more favourable silane compound for the construction of interfaces on HFET sensors. We also show that wear of APDMES interfaces on silicon/ SiO_2 is proportional to normal load on cantilevers, as expected. Interface removal with mechanical insult is not a concerted process, but rather increasing amounts of material are removed as the load increases. Despite the apparent lubricating properties of streptavidin, at all loads, streptavidin-bound polymeric interfaces wore the most deeply, owing to the so-called cushioning effect. *In toto*, these data corroborate expected (based on chemistry and deposition protocol), differential properties (thickness, durability, etc.) of the interface layers based on silane polymers on SiO_2 and Al_2O_3 . The findings suggest that, when used to build sensing interfaces of planar FET protein sensors operating in physiological buffer, since FET signals scale strongly with the distance between the analyte charges and the sensing surface, of the interfaces tested, those based on APDMES should give the highest magnitude sensing signal, assuming identical numbers of analyte molecules bound, and should be most resistant to abrasive insult.

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