Oxide-Dependent Adhesion of the Jurkat Line of T Lymphocytes

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The adhesion force of Jurkat cells was measured using atomic force microscopy (AFM) in aqueous solution at pH 7.2 on six metal oxide surfaces, namely, two quartz (α-SiO2) crystal faces, amorphous SiO2 glass, rutile (α-TiO2), muscovite mica (KAl3(AlSi3O10)(OH)2), and polycrystalline corundum (α-Al2O3). We show quantitatively for the first time that the T lymphocyte adhesion force and adhesion work correlates with substrate point of zero charge, indicating greater adsorption on surfaces with smaller negative charge. Adhesion events also exhibited sawtooth-shaped force–distance profiles indicative of protein bonds. No significant correlations were found with oxide Hamaker constants, indicating negligible contributions from van der Waals forces, nor with surface roughness. These results suggest that, when cell–surface receptors are not activated, Jurkat cell adhesion is dominated by specific interactions related to the unfolding of modular glycoproteins or other proteins that are not unique to T-cell surfaces and by electrostatic forces between negatively charged glycoproteins and variably charged oxide surfaces. Our results have implications for the interactions of immune system cells with metal oxides present in the human body either by design as in biomedical applications or inadvertently such as inhaled mineral dust particles in the lung.

Introduction

Immune system cells are the first responders to foreign objects in the human body, including bacteria, viruses, fungi, foreign proteins, inhaled mineral dust particles, and implanted prosthetic devices. Oxide- and silicate-based ceramic biomaterials such as titania and bioactive glass are used as prosthetic implants in the human body. Aluminum and titanium metal-based orthopedic implants develop a surface layer of the corresponding oxide when exposed to air or solution. Metal oxides are also used as substrates for supported lipid bilayers in applications such as microfluidic and molecular recognition devices and biosensors. Furthermore, nanoparticulate metal oxides are being developed for tissue engineering applications and for encapsulated drug and gene delivery systems.

Metal oxides may also enter the body inadvertently as inhaled dust particles from wind-blown dusts, construction, blasting, and mining activities. In addition to factors such as exposure time and dose, the fate of inhaled mineral dust particles such as quartz, asbestos, and coal in the lung and their potential for causing respiratory disorders depend on the mineral's crystal surface chemistry. The reactivity of surface bonds is ultimately controlled by the oxide's chemical composition, crystal structure, and crystal face orientation.

Whether the oxides are present in the human body by design or by accident, it is important to know how the unique surface chemistry of each oxide will control the initial interactions with cells, especially those of the immune system. The motivation of the present work was to determine whether oxide surface chemical properties (chemical composition, structure, crystal face orientation, point of zero charge, and Hamaker constants) and physical properties (surface roughness) affect the adhesion of the T lymphocyte, a type of immune system cell, and to determine the forces controlling adhesion. Adhesion strength was measured using atomic force microscopy (AFM), which can record interaction forces in the piconewton range.

When a pathogen enters the body, a complex set of nonspecific and specific immune system responses occur. In very general terms, the pathogen is engulfed and destroyed by phagocytic cells including macrophages and neutrophils. Mature B cells secrete antibodies that bind to the invader and signal macrophages and other cells to engulf and destroy the invader. Killer T cells can directly recognize and kill pathogen-infected cells in the body or can recognize the pathogen and secrete lymphokines that stimulate B cells, killer T cells, and macrophages.

Although macrophages come into direct contact with the pathogen, these cells have a very irregular shape, so they are not ideal candidates for adhesion force studies using AFM. Therefore, we have used the Jurkat line of T lymphocytes as a model for immune cells that may come into contact with the surfaces of foreign objects in the human body. Jurkat cells are an ideal model system because T lymphocytes have a spherical shape, and their stiffness of 48 ± 34 Pa on a glass substrate is close to that of alveolar macrophages with a stiffness of 43 ± 1.9 Pa. Also, an experimental protocol already exists in the literature for the attachment of the Jurkat line of T cells to AFM cantilevers.

(9) Sahai, N.; Schoonen, M. A. A.; Skinner, H. C. W. In Medical Mineralogy and Geochemistry; Reviews in Mineralogy and Geochemistry Series; Sahai, N., Schoonen, M. A. A., Eds.; Mineralogical Society of America: Washington, DC, 2006; Vol. 64, pp 1–4.
The oxide supports that we used were an amorphous silica glass slide (a fused quartz plate), the (101) and (100) crystal faces of natural quartz (α-SiO₂), the (001) cleavage face of natural muscovite mica, the (100) face of rutile (α-TiO₂), and a polycrystalline, randomly oriented corundum (α-Al₂O₃) substrate. These oxides were chosen because of their range of surface properties in terms of chemical composition, crystal structure, and crystal face orientation, which are ultimately related to the oxide’s characteristic point of zero charge (PZC). The PZC is the pH at which the oxide surface has a net zero surface charge, thus controlling the electrostatic bonding forces. The substrates chosen here represent a wide range of PZCs as well as a range of Hamaker constants, which are proportional to the van der Waals bonding force. The substrates chosen here have been used extensively as a substrate in studies of oxide-supported lipid bilayers because mica has perfect cleavage along the (001) face resulting in an atomically smooth surface (Table 1).

The adhesion strength was estimated in terms of the detachment force (Fdet), defined as the maximum force used to remove the Jurkat cell from the oxide surface. We also determined the detachment work (wdet) required to remove the Jurkat cell from the oxide surface. Adhesion strength results were interpreted in terms of binding to cell surface receptors and glycoproteins via specific, electrostatic, and van der Waals bonds.

### Methods

**Materials.** All water used was deionized and purified to a resistivity of 18.2 MΩ·cm (Barnstead Nanopure Diamond purifier). Buffer constituents, namely, enzyme-grade HEPES (Rolph Scientific, Covina, CA), metal specimen disks (“pucks”) for AFM (15 mm diameter of magnetic stainless steel, alloy 430, Ted Pella, Inc., Redding, CA), and tipless, pyramidal-shaped cantilevers (product name NT) with a manufacturer-provided spring constant of 0.06 N·m⁻¹ and a vibrational frequency of 12 kHz (Veeco, Woodbury, NY) were used. All AFM measurements were made on a Digital Instruments/Veeco MultiMode microscope with a Nanoscope IV controller.

**Cell Growth.** Jurkat clone E6-1 was maintained in RPMI 1640 growth medium containing 2 mM l-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1 mM sodium pyruvate and supplemented with 10% fetal bovine serum. Cells were grown in a 37 °C humidifying incubator at 5% CO₂ and split every 4 days.

**Substrate Cleaning.** The oxides were first rinsed in acetone and then DI water. Subsequently, the oxides were sonicated for 15 min in a 2% Hellmanex II solution (Hellma Cells, Inc.), which is an alkaline cleaning solution specifically designed to clean metal oxide cudevets. This was followed by a rinse in DI water, sonication for 5 min in acetone, a DI water rinse, sonication for 5 min in DI water, and a final DI water rinse. Mica was freshly cleaved with a piece of adhesive tape before each use and did not require any cleaning. Oxide slabs/windows were attached to the AFM metal pucks using a sticky adhesive.

**Cantilever Spring Constants.** The dimensions of the cantilevers were measured using a Micromat 2003 Microhardness Tester. Cantilever spring constants were determined experimentally with the Sader method using a PCM-90 probe calibration module (Novascan Technologies, Inc., Ames, IA).

**Concanavalin-A Cantilever Functionalization.** To attach a Jurkat cell to the cantilever, a tipless silicon nitride cantilever was functionalized using a modified version of a previously published procedure. The cantilever was soaked in acetone for 10 min, washed with DI water, and then dried overnight in a 50 μL drop of biotin-BSA (0.5 mg·mL⁻¹ in 0.1 M sodium bicarbonate buffer) at 37 °C in an improved homebuilt humidified incubator. The cantilever was then washed with 5 mL of PBS (0.01 M phosphate, 0.138 M NaCl, 0.0027 M KCl, pH 7.4) and placed in 1.5 mL of a 1% glutaraldehyde solution for 30 s. Next, the cantilever was washed with 5 mL of PBS, incubated in a 50 μL drop of streptavidin (0.5 mg·mL⁻¹ in PBS) at room temperature for 10 min, washed with 5 mL of PBS, and placed in a 50 μL drop of biotinylated concanavalin A (0.5 mg·mL⁻¹ in PBS) for 10 min at room temperature. Finally, the cantilever was washed with 5 mL of PBS and stored at 4 °C in PBS. Although we did not

### Table 1. Properties of Solid Substrates Used

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Crystal face</th>
<th>Point of zero charge (PZC)</th>
<th>Hamaker constant (× 10⁻²⁰ J)</th>
<th>Surface roughness (nm)</th>
<th>Resistivity (Ω·cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>quartz (α-SiO₂)</td>
<td>(101)</td>
<td>2.9±0.19</td>
<td>17.6±22</td>
<td>0.181</td>
<td>3.2×10¹⁷</td>
</tr>
<tr>
<td>quartz (α-SiO₂)</td>
<td>(100)</td>
<td>2.7±0.19</td>
<td>17.6±22</td>
<td>0.729</td>
<td>3.2×10¹⁷</td>
</tr>
<tr>
<td>amorphous silica (SiO₂)</td>
<td>none</td>
<td>3.5±0.16</td>
<td>8.0±22</td>
<td>0.206</td>
<td>3.2×10¹⁷</td>
</tr>
<tr>
<td>rutile (α-TiO₂)</td>
<td>(100)</td>
<td>5.9±0.20</td>
<td>54.2±23</td>
<td>0.076</td>
<td>3.2×10¹⁷</td>
</tr>
<tr>
<td>mica, muscovite</td>
<td>(001)</td>
<td>3.6±0.16</td>
<td>11.8±23</td>
<td>0.041</td>
<td>3.2×10¹⁷</td>
</tr>
<tr>
<td>corundum (α-Al₂O₃)</td>
<td>polycrystalline</td>
<td>9.4±0.21</td>
<td>24.6±22</td>
<td>33.0±23</td>
<td>0.107</td>
</tr>
</tbody>
</table>
conduct any tests to confirm the viability of the cells attached to the tips, the original published protocol that we followed implies that the cells were alive on the AFM tips.12

Deflection–Distance Curves with the Jurkat Cell and Concanavalin-A. The cantilever was moved toward the cell and gently pressed onto the chosen cell (Figure 1). Contact with the cell could be monitored by the change in voltage on the photodiode output signal. The cantilever was left in contact with the cell for 30–60 s before being pulled away from the substrate. At this point, the cell was no longer in the field of view and was assumed to be bonded to the cantilever. The fluid cell was removed from the AFM head, and a drop of HEPES buffer solution was injected through the input line of the fluid cell so that the cell-functionalized cantilever would not be exposed to air.

The oxide puck was placed onto the AFM scanner, and the fluid cell was placed back in the AFM head. The cantilever was engaged in contact mode with a 1 nm scan size, and the AFM was switched to force mode (1 μm ramp size, 0.99651 Hz rate, 2.5 V data scale). Deflection–distance curves between a Jurkat cell and each oxide were obtained. Three cell-functionalized tips were used, and five different locations with each cell were examined, on each oxide. Several tens of data sets were taken at every position on the oxide, resulting in hundreds of deflection–distance curves on each oxide in order to obtain statistically significant data. A larger number of data sets were obtained for quartz (101), quartz (100), and polycrystalline corundum because these surfaces are rougher than those of rutile (100) and mica (001) and also because the former oxides represent the extremes of PZC (Table 1).

Images of a Jurkat cell adhered to the cantilever tip could not be obtained despite our best attempts. Therefore, we obtained deflection–distance curves for a concanavalin-A (con-A)-functionalized cantilever (without an attached cell) and the substrates. The con-A deflection–distance curves were considered to be blank runs.

Sample deflection–distance curves for each oxide are shown in Figure 2. It is evident that the shapes of the curves for the cell-functionalized tips are very different from the con-A-functionalized blank runs. In addition to the qualitative shape of the curve, a quantitative measure of the difference between cell-functionalized and con-A-functionalized tips is provided by the sensitivity in each case. The sensitivity is the slope of the deflection–distance curve in the constant compliance region when the substrate and tip are coupled and moving together. Sensitivity values for cell-functionalized versus con-A-functionalized tips are shown in Supporting Information Figure 1. A difference in sensitivity of the cell-functionalized versus con-A-functionalized tips suggests that a cell was, in fact, attached to the tip. Clearly, the cantilever sensitivity is greater for the cells than for the tips, and the maximum deflection is generally greater for the con-A-functionalized tips. These results confirm that a cell was attached to the tip in each case.

Variations in deflection–distance curves for a given oxide substrate may occur because of potential spatial variations in surface roughness and chemical composition on the oxide surface.24 More likely causes are differential cell deformation at the oxide surface and/or differences in the contact force (the force of the cantilever tip pressing onto the oxide surface, \( F_{\text{det}} \)). Jurkat cells are deformable (stiffness of 48 ± 35 Pa)13 and are increasingly deformed and flattened as they are pressed into the substrate with an increasing tip contact force. As a result, the contact area between the cell and substrate increases, which corresponds to an increase in the detachment force as reported in previous studies.25 Therefore, deflection–distance curves were collected within a contact force range of 9500–12 500 pN.

Data Analysis and Force–Distance Curves. The raw deflection piezo data were converted from binary to ASCII files and then into force–distance curves using the AFM software. The force–distance data were analyzed using a computer program written by Christopher Muffles (Department of Geology and Geophysics, University of Wisconsin, Madison) for the maximum cell detachment force \( (F_{\text{det}}) \).26 Cell detachment work \( (w_{\text{det}}) \) was calculated as the area integrated under the force–distance curve.

Results

The deflection–distance curves for cell detachment vary significantly from one oxide to another (Figure 2). During tip retraction, many of the curves show a “sawtooth” pattern with a variable number of teeth for the different oxides. The mean values of \( F_{\text{det}} \) and \( w_{\text{det}} \) obtained from hundreds of measurements for each oxide are summarized in Tables 2 and 3, respectively, for Jurkat cells and con-A-functionalized tips. The mean \( F_{\text{det}} \) values increased in the sequence quartz (100) < silica glass < quartz (100) < quartz (100) < silica glass < quartz (100) < silica glass < quartz (100) < quartz (100) < polycrystalline corundum (Table 2). The \( F_{\text{det}} \) range of \( \sim 350–900 \) pN measured here compares well with the adhesion force of \( \sim 320 \) pN between T and B lymphocytes,27 the detachment force of \( \sim 100–600 \) pN between bacteria and macrophages,28 and the detachment force of \( \sim 100–400 \) pN needed to pull apart two cells that display antigen–ligand pairs.29

The mean \( w_{\text{det}} \) shows the same general oxide-dependent trend as \( F_{\text{det}} \). The range of \( w_{\text{det}} \) \( \sim 73–150 \times 10^{-18} \) J measured in the present work compares favorably with the detachment work of \( \sim 200–800 \times 10^{-18} \) J between two Jurkat cells25 and between two cells that display antigen–ligand pairs.

In addition to the oxide-dependent trends in mean \( F_{\text{det}} \) and \( w_{\text{det}} \), quite large ranges were observed for a given oxide (Tables 2 and 3), as discussed above in the Methods section. The variability in \( F_{\text{det}} \) and \( w_{\text{det}} \) is summarized as range, mean, and modal values in Tables 2 and 3, respectively, for Jurkat cells and con-A-functionalized tips. The \( F_{\text{det}} \) and \( w_{\text{det}} \) data for Jurkat cells are shown as histograms (Figures 3 and 4). Despite the variability for a given oxide, it is clear that the shapes of the histograms are different from one oxide to another. In detail, the \( F_{\text{det}} \) and \( w_{\text{det}} \) values exhibit a Gaussian unimodal distribution for quartz (100),

Figure 1. Image of Jurkat cells on a mica substrate close to a 196-μm-long AFM cantilever. The field of view is \( \sim 612 \times 465 \) μm².
Table 2. Detachment Force and Detachment Work for the Interaction of Jurkat Cell-Functionalized Tips with Substrates of Different Chemical Composition, Crystal Orientation, and Surface Roughness

<table>
<thead>
<tr>
<th>Surface</th>
<th>Surface Roughness (nm)</th>
<th>$F_{\text{det}}$ (pN)</th>
<th>Modal $F_{\text{det}}$ (pN)</th>
<th>Mean $w_{\text{det}}$ ($10^{-18}$ J)</th>
<th>Modal $w_{\text{det}}$ ($10^{-18}$ J)</th>
<th>No. of Curves (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz (101)</td>
<td>0.181</td>
<td>548 ± 347</td>
<td>300–350</td>
<td>124 ± 48</td>
<td>120–130</td>
<td>433</td>
</tr>
<tr>
<td>Quartz (100)</td>
<td>0.729</td>
<td>352 ± 278</td>
<td>200–250</td>
<td>74 ± 56</td>
<td>60–70</td>
<td>1079</td>
</tr>
<tr>
<td>Silica</td>
<td>0.206</td>
<td>501 ± 339</td>
<td>250–300</td>
<td>70 ± 46</td>
<td>30–40</td>
<td>137</td>
</tr>
<tr>
<td>Rutile (100)</td>
<td>0.076</td>
<td>587 ± 361</td>
<td>300–350</td>
<td>116 ± 70</td>
<td>60–70</td>
<td>100</td>
</tr>
<tr>
<td>Mica (001)</td>
<td>0.041</td>
<td>708 ± 150</td>
<td>600–650</td>
<td>111 ± 39</td>
<td>100–110</td>
<td>265</td>
</tr>
<tr>
<td>Polycrystalline Corundum</td>
<td>0.107</td>
<td>878 ± 546</td>
<td>150–200</td>
<td>138 ± 87</td>
<td>140–150</td>
<td>859</td>
</tr>
</tbody>
</table>

*a* Standard deviations are provided for mean $F_{\text{det}}$ and mean $w_{\text{det}}$.

Figure 2. Sample deflection-piezo AFM curves between a Jurkat cell (red) or a con-A-functionalized tip (blue) and the following substrates: (a) quartz (101), (b) quartz (100), (c) silica, (d) rutile (100), (e) mica (0001), and (f) polycrystalline corundum.
a bimodal distribution for amorphous silica glass and quartz (101), and a multimodal distribution for rutile (100), mica (001), and polycrystalline corundum. The number of modes generally agrees with the number of sawtooth points observed in the cell detachment–distance curves (Figure 2).

**Discussion**

The oxide-dependent trends of mean $F_{\text{det}}$ and $w_{\text{det}}$, noted above, suggest that Jurkat cell adhesion may depend on a number of substrate chemical and physical properties such as chemical composition, structure (e.g., silica glass vs crystalline quartz), crystal face orientation (e.g., quartz (101) vs (100)), Hamaker constant, and surface roughness (e.g., quartz (101) vs quartz (100) and silica glass). In addition, the molecules present on the cell surface will influence the types of specific and nonspecific bonds formed. Each of these factors will be considered below in detail.

The sawtooth shape observed in many of the retraction curves suggests the breakage of specific bonds between cell surface ligands such as specific receptors, and (glyco)proteins, and the oxide surface. This inference is consistent with peaks (modes) in $F_{\text{det}}$ and $w_{\text{det}}$ histograms as the detachment of particular surface ligands and receptors from the substrate surface.27,28,30 Alternatively, the sawtooth pattern may represent the breaking of

<table>
<thead>
<tr>
<th>surface</th>
<th>surface roughness (nm)</th>
<th>mean $F_{\text{det}}$ (pN)</th>
<th>modal $F_{\text{det}}$ (pN)</th>
<th>mean $w_{\text{det}}$ ($10^{-18}$ J)</th>
<th>modal $w_{\text{det}}$ ($10^{-18}$ J)</th>
<th>no. of curves (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>quartz (101)</td>
<td>0.181</td>
<td>473 ± 296</td>
<td>100–150</td>
<td>56 ± 25</td>
<td>30–40</td>
<td>125</td>
</tr>
<tr>
<td>quartz (100)</td>
<td>0.729</td>
<td>253 ± 79</td>
<td>200–250</td>
<td>65 ± 7</td>
<td>60–70</td>
<td>50</td>
</tr>
<tr>
<td>silica</td>
<td>0.206</td>
<td>493 ± 208</td>
<td>300–350</td>
<td>24 ± 10</td>
<td>20–30</td>
<td>93</td>
</tr>
<tr>
<td>rutile (100)</td>
<td>0.076</td>
<td>6055 ± 470</td>
<td>6100–61500</td>
<td>1157 ± 84</td>
<td>1120–1130</td>
<td>108</td>
</tr>
<tr>
<td>mica (001)</td>
<td>0.041</td>
<td>282 ± 106</td>
<td>200–250</td>
<td>64 ± 18</td>
<td>40–50</td>
<td>371</td>
</tr>
<tr>
<td>polycrystalline corundum</td>
<td>0.107</td>
<td>1166 ± 321</td>
<td>1000–1050</td>
<td>351 ± 92</td>
<td>330–340</td>
<td>226</td>
</tr>
</tbody>
</table>

*Standard deviations are provided for mean $F_{\text{det}}$ and mean $w_{\text{det}}$.

**Figure 3.** Histograms of detachment force between a Jurkat cell and the following substrates: (a) quartz (101), (b) quartz (100), (c) silica, (d) rutile (100), (e) mica (001), and (f) polycrystalline corundum.

sacrificial bonds (intramolecular or molecule–oxide) and the opening up of hidden length associated with the unraveling of a looped protein, where each sawtooth represents the unfolding of a loop.31,32 These interpretations are supported by the data in our study, where the number of modes in the histograms corresponds approximately to the number of teeth in the tip-retraction curves. Furthermore, it has been estimated that the unfolding of loops in a protein requires about 2 orders of magnitude more energy ($w_{\text{det}} \approx 1 \times 10^{-17}$ J) than the sacrificial breakage of bonds ($w_{\text{det}} \approx 1 \times 10^{-19}$ J).31–33 The values of $w_{\text{det}}$ measured in our study fall within the range for unfolding loops on a protein.

The question that naturally follows is the identity of the protein involved in adhesion. A complete characterization of the cell surface receptors on the Jurkat cells under our experimental conditions requires extensive immunological studies and is well beyond the scope of the present study. Nonetheless, we will address below the potential receptors and ligands involved in adhesion and detachment and infer a plausible candidate molecule involved in specific binding.

We begin with potential candidate molecules on the Jurkat cell surface that may be involved in specific binding to the oxide substrates. In general, there are four types of proteins that are not unique to lymphocytes that dominate cell–cell adhesion: cadherins, selectins, the immunoglobulins, and integrins.34,35 These proteins require complementary binding sites (i.e., biomolecular receptors) on the substrate to mediate cellular adhesion,36 but our oxide substrates lack these sites. Furthermore, adhesion due to cadherins and selectins is dependent on Ca$^{2+}$, which was not present in our solutions. Therefore, we can eliminate cadherins, selectins, immunoglobulins, and integrins as plausible proteins responsible for the sawtooth pattern observed in the deflection–distance curves (Figure 1). In addition to these proteins that are not unique to lymphocytes, activated T lymphocytes display specific cell surface proteins such as lymphokines and proteases that are activated only if the substrate has the complementary antigens. Our oxide substrates lacked these sites. Furthermore, adhesion due to cadherins and selectins is dependent on Ca$^{2+}$, which was not present in our solutions. Therefore, we can eliminate cadherins, selectins, immunoglobulins, and integrins as plausible proteins responsible for the sawtooth pattern observed in the deflection–distance curves (Figure 1). In addition to these proteins that are not unique to lymphocytes, activated T lymphocytes display specific cell surface proteins such as lymphokines and proteases that are activated only if the substrate has the complementary antigens. Our oxide substrates lacked these sites.

![Figure 4. Histograms of detachment work between a Jurkat cell and the following substrates: (a) quartz (101), (b) quartz (100), (c) silica, (d) rutile (100), (e) mica (001), and (f) polycrystalline corundum.](image-url)

deemed unlikely. Specific interactions of Jurkat cells with oxides in the present study may, then, be mediated by glycoproteins or some other nonunique protein on the cell surface.

Glycoproteins have a net negative charge due to the presence of sialic acid moieties34,37,38 and could confer a negative charge on the Jurkat cell surface, consistent with the negative zeta potential measured on T lymphocytes by electrophoretic mobility.37 Accordingly, electrostatic interactions are possible between the Jurkat cell and the charged oxide surfaces. The magnitude and sign of the electrostatic charge on the oxide can be estimated from characteristic crystal-chemical properties of the oxide.

Oxides have amphoteric hydroxyl surface sites that can adsorb or desorb protons, resulting in net positive, neutral, or negative surface charge. The protonation and deprotonation reactions of surface hydroxyls can be described by equilibrium reactions and their corresponding equilibrium acidity constants.13–18 Each oxide has a characteristic pH known as the point of zero charge (PZC), where the net surface charge is zero. The PZC is related to the sum of the surface acidity constants. Oxide surfaces are positively charged at pH < PZC and negatively charged at pH > PZC. The PZC of an oxide depends on its chemical composition, crystal structure, crystal orientation, and interfacial hydration.17,18

The two quartz surfaces and silica have PZCs of ~2–4 and rutile (100) has a PZC of ~5.9, so their surfaces are negatively charged at the experimental pH of 7.2 (Table 1). In contrast, the randomly oriented polycrystalline corundum with PZC = 9.4 has a positive surface charge. Muscovite mica (001) has a slight negative surface charge (PZC = 6.6).

The relationship of adhesion strength with oxide surface charge is shown in Figures 5a and 6a for Jurkat cells and con-A-functionalized tips. A strong linear correlation is obtained for Jurkat cells, indicative of electrostatic forces contributing to the overall adhesion strength. Cell adhesion strength increases as the magnitude of negative surface charge decreases and positive surface charge increases from quartz and silica glass to rutile (100), mica (001), and polycrystalline corundum. In contrast, the con-A-functionalized tips exhibit no correlation with oxide surface charge. This can be explained by the fact that con-A has an isoelectric point of ~7.2,39,40 which is identical to the experimental pH, so it is neutrally charged in our experiments.

van der Waals forces are generally attractive and operate when colloid particles, including cells, approach within ~3–5 nm of each other.41–44 The relative magnitude of van der Waals forces between two objects in a medium depends on the Hamaker constants of each object in the medium.45 The Hamaker constant of the cell and of con-A are constant for each oxide–water system, so we can use the Hamaker constant of oxides in an aqueous medium (Table 1). The mean $F_{\text{det}}$ and $w_{\text{det}}$ of the cells showed no correlation with the Hamaker constants (Figures 5b and 6b), indicating a negligible contribution of van der Waals forces to adhesion. In contrast, con-A tips appear to attach to the oxide surface by van der Waals forces, indicated by the strong linear correlations of their mean $F_{\text{det}}$ and $w_{\text{det}}$ with Hamaker constants.

In addition to the chemical properties, physical properties such as surface roughness may also affect $F_{\text{det}}$ (e.g., quartz (101) vs quartz (100) and silica glass). A higher surface roughness limits the contact area between the cell and substrate, resulting in a lower

Figure 5. Cell adhesion force for Jurkat cells (red circles) and con-A-functionalized AFM tips (blue inverted triangles) as a function of oxide properties: (a) oxide PZC, (b) Hamaker constant, and (c) surface roughness.
Different adhesion models exist in the literature for relating the detachment force to the work per unit surface area, $F_{\text{det}}$, for a sphere of radius $R$ attached to a substrate of Hamaker constant, $H$, according to $^{45-47}$

$$F_{\text{det}} = -A\pi R\gamma_{\text{det}}$$  

(1)

where $A = 3$ or 4 according to the model. Using the cantilever length as a scale (Figure 1), the radius of the Jurkat cell is $\sim 10 \mu m$, and combined with the experimental mean $F_{\text{det}}$ values (Table 2), we have calculated theoretical $\gamma_{\text{det}}$ values of $0.002$–$0.009 \text{ mJ/m}^2$ for the various oxides (Supporting Information Table 1). These values are within the range $(0.001–1 \text{ mJ/m}^2)$ that is representative of dominant van der Waals forces $^{41,48}$. However, this calculation assumes that the whole cell is in contact with the oxide.

If contact is via (glyco)proteins, then the experimental mean $w_{\text{det}}$ values (Table 2) should be related to the number of (glyco)protein binding sites per unit surface area. Site-density ranges of $1/100 \text{ nm}^2$ $^{48}$ to $1/1000 \text{ nm}^2$ $^{49}$ are considered to be reasonable for most adhesion molecules, which results in $\gamma_{\text{det}}$ values, respectively, of $700$–$1400$ and $70$–$140 \text{ mJ/m}^2$ (Supporting Information Table 1). A final calculation involves the use of Hamaker constants and the closest distance of approach ($D$) between the oxide and the cell surface. If a (glyco)protein mediates the attachment and $D = 2.5 \text{ Å}$ corresponds to the thickness of a water monolayer between the (glyco)protein and oxide substrate, then the calculated adhesion work due to van der Waals forces ranges ($\gamma_{\text{ads}}$) from $53$ to $359 \text{ mJ/m}^2$ (Supporting Information Table 1). Both of these sets of calculations yield adhesion work values that are too large to be due to van der Waals forces and are representative of adhesion dominated by other forces. $^{48}$ We propose that the dominant forces are the unraveling of loops on the modular (glyco)proteins and electrostatic forces between the glycoprotein and oxide surface. Complex immunological/genetic studies to confirm our inferences are beyond the scope of the present work, where the goal was to determine if the oxide surface properties contribute to cell adhesion. Nevertheless, the empirical correlations and detailed model analyses conducted here strongly suggest that oxide surface charge and the unraveling of cell surface (glyco)proteins are responsible for the observed detachment force and work on different oxides.

**Conclusions**

We have demonstrated quantitatively for the first time that, in the absence of activated cell surface receptors and complementary molecules on oxide surfaces, T lymphocyte adhesion on oxides is controlled predominantly by specific interactions involving (glyco)protein unfolding and electrostatic-dominated bonds between glycoproteins and oxide surfaces. The oxide surface charge (PZC) is determined by chemical properties such as the chemical composition, crystal structure, and crystal face orientation.

The present study represents a “whole-cell, top-down” approach to studying human cell surface interactions with oxides. Bacterial cells, although generally much smaller than the present Jurkat cells, seem to show similar adhesion behavior. Glycoproteins mediate cell–cell adhesion, $^{50}$ and the detachment work from...
oxide surfaces depends on PZC. Furthermore, we have previously used oxide-supported lipid bilayers in a “model bilayer, bottom-up” approach to examine cell membrane—oxide interactions. Interestingly, we found that the adsorption and rupture of dipalmitoylphosphocholine (DPPC) vesicles also depends on the surface charge of the oxide and on van der Waals interactions. Thus, oxide surface chemistry controls cell—surface interactions from the nanometer scale of phospholipid vesicles and bilayers to the micrometer scale of microbial and human cells, and the results can be sensibly interpreted within the framework of physical—chemical models of adhesion. Such studies have implications for understanding the interactions of immune system cell surfaces with bioceramic implants as well as bacterial cell interactions with minerals in the natural geological environment.

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Supporting Information Available: Sensitivities for cell versus con-A-functionalized tips of sample AFM deflection—distance curves. Calculation of detachment work per unit surface area and work of adhesion due to van der Waals forces. This material is available free of charge via the Internet at http://pubs.acs.org.


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