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Comparative Properties of Siloxane vs Phosphonate Monolayers on A Key Titanium Alloy

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A direct comparison of surface loading, interface shear strength, and interface hydrolytic stability was made between a phosphonate and two siloxane monolayers formed on the native oxide surface of Ti-6Al-4V. Surface loading for the phosphonate was ca. four times greater (on a nanomole/area basis) than for the siloxanes; mechanical strengths per surface-bound molecule were comparable, but the hydrolytic stability (pH 7.5) of the siloxanes was poor. These results suggest that phosphonate monolayer interfaces are more desirable than comparable siloxane ones for applications where such interfaces contact even slightly alkaline water.

Chemically modified oxide surfaces are intrinsic components of materials finding broad application in, for example, chromatography, corrosion inhibition, adhesion, sensors, and even biomedical devices[1,2] and have been prepared on a wide variety of substrates such as silica,[2−4] various ceramics,[5,6] and structural metals, including titanium.[7−10] A commonly used methodology to form surface organic coatings on oxide surfaces is based on siloxane chemistry in which chloro-, amino-, or alkoxysilane "head groups" are used to attach an organic "tether" to the oxide surface.[3,11−15] This process occurs via proton transfer from surface hydroxyl (OH) sites and can give a surface-bound monolayer of siloxane under ideal conditions. However, such monolayer formation often competes with lateral cross-linking; if the number of available surface OH groups is limited, low yields of direct surface attachment can result[9] and siloxane cross-linking can predominate over surface attachment.[10,16,17] Where functionalized silanes such as aminopropyl(triethoxy)silane (APTES) are used, a further complication is that the amino "tail group" can also coordinate to the surface.[5,18] Finally, even after siloxane coating has been achieved, the hydrolytic lability of the surface oxygen−silicon bond, especially under alkaline conditions, could further compromise this technology.[1,6,9,10] We have described a new method to bond organics to various oxide-coated surfaces using phosphonate interfaces; unlike siloxanes, phosphonate interface synthesis is not limited by low native oxide surface OH group content. We are interested in developing stable interfaces to bond biomolecules to native oxide-coated metals[9,19,20] which must be stable to physiological pH. Therefore, we have quantitatively assessed, side by side, derivatized siloxane and phosphonate interfaces on the medically important alloy Ti-6Al-4V and we have measured their surface loadings, mechanical strengths, and hydrolytic stabilities.

Three methods were used to derivatize Ti-6Al-4V. The first involved silanization using APTES (**1**) followed by standard organic procedures, for example, by activation using a maleimido coupling reagent, to bond a fluorophore to the surface. However, since APTES can react with an oxide at either its amine or siloxane terminus,[5,18] a second method was developed to obviate this complication. This involved preparing "pre-activated" (3-triethoxysilylpropyl)-6-*N*-maleimidohexanamide (**2**) in solution, attaching **2** to the alloy surface, and then bonding the fluorophore. The third method involved bonding a hydroxyl-terminated phosphonate to the alloy surface by self-assembly/heating[21] (see Scheme 1), which forms a covalently bound surface phosphonate film likely by reaction first with surface Ti−OH and then with Ti−O−Ti groups.[22] Surface coverage by each interface was measured directly using our quartz crystal microgravimetry (QCM) technique.[4,9] The shear strength of each was also measured, mechanically, according to ASTM test F-1066.[22] Finally, the stability to hydrolysis of each fluorophore-labeled interface was measured by fluorescence spectroscopy.[20] We find that initial surface coverages

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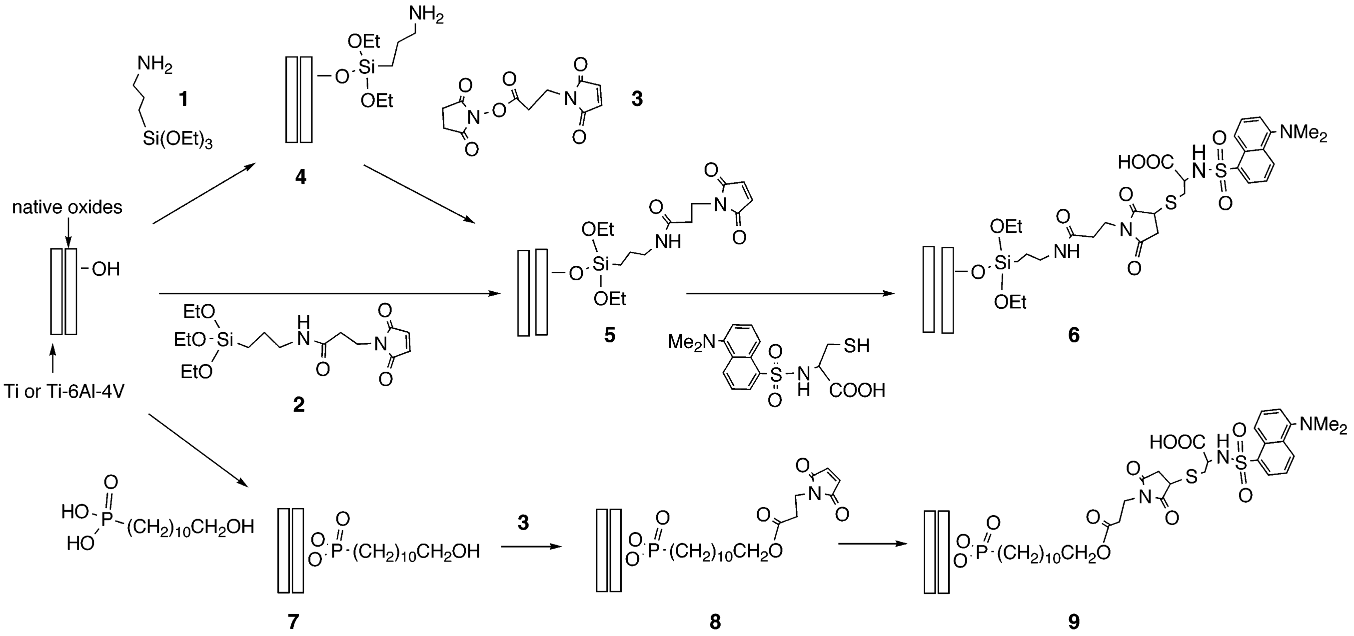
Scheme 1. Synthesis of Fluorophore-Labeled Interfaces



by either siloxane are lower than that by the phosphonate; the mechanical strengths of each interface are comparable. However, the hydrolytic instability of the siloxane interfaces determined under physiological conditions contrasted sharply with the phosphonate interface, which was robust.[9]

Experimental Section

General. Aminopropyl(triethoxy)silane (**1**) (Gelest), 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (**3**) (Aldrich, 99%), *N*-(5-(dimethylamino)-1-naphthylsulfonyl)-cysteine DCHA salt (DANSYL-cysteine, Research Plus), and thionyl chloride were used as received. Acetonitrile, toluene, and triethylamine were distilled prior to use. Disks of Ti-6Al-4V ELI (3/8 in. diameter) were sanded first with 240 grit and then 500 grit silicon carbide paper, rinsed with sonication successively with methylene chloride, methyl ethyl ketone, and methanol, dried, and stored in an oven at 200 °C. All surface films were analyzed using a Midac M2510C interferometer equipped with a Surface Optics SOC4000 SH specular reflectance head attachment. Fluorimetry measurements were made using a Jobin Yvon Fluorolog 3 spectrometer. 11-Hydroxyundecylphosphonic acid was synthesized as previously reported.[23]

(3-Triethoxysilylpropyl)-6-*N*-maleimidohexanamide (2). *N*-Maleimidocaprionoyl chloride was prepared by stirring 0.5 g of *N*-maleimidocaprionic acid in 40 mL of toluene with 0.38 mL of thionyl chloride. The reaction proceeded under N_2 at 70 °C for 5 h. The solvent was removed to give yellow, oily maleimidocaprionoyl chloride which was then dissolved in 10 mL of toluene containing 1 mL of triethylamine and 0.48 mL of APTES. The mixture was stirred at room temperature under N_2 for 36 h. Excess solvent was evaporated to give a viscous yellow oil that was identified by ^{1}H NMR: ($CDCl_3$) δ 0.6 (m, C**H**$_2$Si, 2H), 1.2 (m, Si(OCH$_2$C**H**$_3$)$_3$, 9H), 1.6 (m (br), alkyl H, 6H), 2.10 (t, C**H**$_2$-CONHCH$_2$, 2H), 3.3 (m, CH$_2$CONHC**H**$_2$, 2H), 3.5 (m, CH$_2$ α to maleimide ring, 2H), 3.9 (m, Si(OC**H**$_2$CH$_3$)$_3$, 6H), 6.7 (s, maleimide H, 2H).

Bonding DANYSL-Cysteine to Ti-6Al-4V ELI via an APTES Interface (5). Disks of Ti-6Al-4V ELI were cleaned as described above, and a film of APTES was deposited using the "T-BAG" technique.[4] The disks were held vertically in a solution of **1** (0.01 mM in THF), and the solvent was allowed to evaporate slowly over 3 h until the level of the solution fell below the disks. The disks were then solvent rinsed with sonication to give surface-bound APTES interface **4** (IR: $\nu_{CH_2} = 2925, 2860\ cm^{-1}$; $\delta_{NH_2} = 1575\ cm^{-1}$ [10]). The disks were then placed in solutions of **3** (0.1 mM in acetonitrile) for 24 h at room temperature then air-dried. They were then rinsed in acetonitrile with sonication and dried in vacuo (0.1 Torr) to give surface-bound **5** (IR: $\nu_{CO_{asymm}} = 1705\ cm^{-1}$). A solution of DANSYL-cysteine (0.3 mM) was prepared in 5 mL of purified water (Millipore), with the pH adjusted to 6.5 using 0.05M NaOH. Disks of **5** were kept in this stirred solution at room temperature for 24 h to give the DANSYLated interface **6**. The disks were then removed from the reaction mixture, rinsed in water with sonication, dried, and analyzed by IR ($\nu_{CO} = 1700, 1650, 1560$ (shoulder) cm^{-1} [10]).

Bonding DANYSl-Cysteine to Ti-6Al-4V ELI Using (3-Triethoxysilylpropyl)-6-*N*-maleimidohexanamide (2). Disks of Ti-6Al-4V ELI were cleaned as described above, and a film of **2** was deposited using the T-BAG technique (from a 0.01 mM solution in THF). The samples were then solvent rinsed with sonication to give surface-bound **5** (IR: $\nu_{CH_2} = 2925, 2860\ cm^{-1}$; $\nu_{NH_2} \approx 3200\ cm^{-1}$; $\delta_{NH_2} = 1575\ cm^{-1}$; $\nu_{CO} = 1772$ (w), $1703\ cm^{-1}$; Figure 1). DANSYL-cysteine was then bound to the surface as described above, also to give **6**.

Bonding DANSYL-cysteine to Ti-6Al-4V ELI *via* an 11-hydroxyundecylphosphonate interface (7). Cleaned disks of Ti-6Al-4V ELI were treated by the T-BAG method with a solution of 11-hydroxyundecylphosphonic acid[23] dissolved in dry tetrahydrofuran (0.1 mM). The disks were then warmed in an oven at 120 °C for 48 h then rinsed with sonication in methanol. Analysis by IR showed an alkyl chain-ordered[24] monolayer film of **7** ($\nu_{CH_{2,asymm}} = 2917\ cm^{-1}$ and $\nu_{CH_{2,symm}} = 2848\ cm^{-1}$) on the alloy surface. Disks of **7** were placed in a solution of **3** in acetonitrile (5 mM) and were kept at room temperature under N_2 for 24 h. The disks were removed from the solution, dried, and analyzed by IR, which shows peaks corresponding to both the maleimide adduct **8** ($\nu_{CO} = 1707, 1734\ cm^{-1}$) and excess **3**; the latter could be removed by rinsing with acetonitrile. The disks were then treated with an aqueous solution of DANSYL-cysteine (1 mM in water, Millipore) at room temperature for 24 h to give **9**, removed from solution, dried, and analyzed by IR ($1650\ cm^{-1}$).

Quartz Crystal Microbalance (QCM) Determination of Surface Loadings on Ti. Piezoelectric quartz crystals (International Crystal Manufacturers [ICM]; AT-cut, 1000 Å Ti electrodes, 10 MHz, overtone polished, 0.201 in. electrode diameter) were used for film deposition and as reference crystals. In each experimental run, the fundamental frequency (f_o) of an untreated QCM was measured. The QCM was then removed

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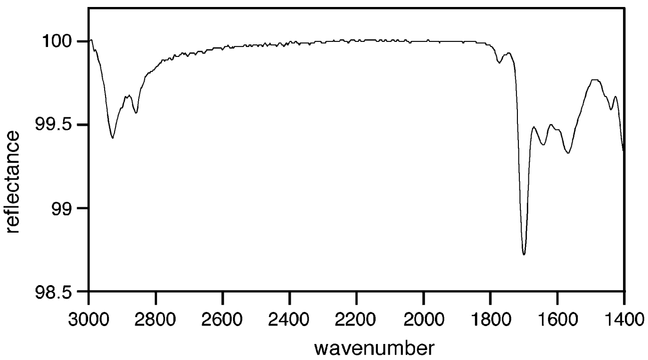

Figure 1. IR spectrum of **6** prepared from **2**.

Table 1. Surface Loading by the Self-Assembled Monolayer Interfacial Films

precursor and interface	loading (nmol/cm^2)
APTES, **4**	0.27 ± 0.01
(3-triethoxysilylpropyl)−6-*N*-maleimidohexanamide, **5**	0.23 ± 0.01
11-hydroxyundecylphosphonic acid, **7**	1.00 ± 0.09

from its holder, and the electrodes on both sides of the crystal were treated with either the silane or phosphonic acid solutions using our previously reported protocol.[4,9] Surface loadings measured by QCM for interfaces formed from APTES (**4**), (3-triethoxysilylpropyl)-6-*N*-maleimidohexanamide (**5**), and 11-hydroxyundecylphosphonic acid (**7**)[9] on Ti are given in Table 1.

Mechanical Testing of Interfacial Shear Strength. Mechanical shear strengths of the three interfaces formed on Ti-6Al-4V were measured according to ASTM test-1066, as previously described.[22] Coupons of polished Ti-6Al-4V were coated pairwise with each of **4**, **5**, and **7**, and the coupon pairs were joined using Cytec Fiberite FM 100 epoxy to achieve an overlap of 2.84 cm^2. After standard curing procedures,[22] the alloy−interface−epoxy−interface−alloy sandwiches were stressed in an Instron model 1331 load cell and the point of interface failure was recorded.

Hydrolytic Stability of Surface-Bound Films Measured by Fluorescence Spectroscopy. Solutions of DANSYL-cysteine (0.1−35.0 μM in Milli-Q water; pH 7.5) were prepared, and a calibration curve was measured. A second calibration curve was measured for analogous solutions at pH 12. Disks of DANSYLated surfaces **6** (prepared by both routes described above) and **9** were placed in cuvettes of Milli-Q water adjusted to pH 7.5 with 0.05 M NaOH. The fluorescence intensities of the supernatants were measured between 10 min and 7 days of immersion. The disks were then removed from solution, dried, and immersed in cuvettes of Milli-Q water adjusted to pH 12 using 0.5 M NaOH for 3 h. The fluorescence intensities of these second supernatants were then measured.

Results and Discussion

We have shown that the T-BAG deposition procedure[4] enables formation of self-assembled monolayers (SAMs) of a broad variety of organophosphonates on the native oxides of Si, Ti, and Ti-6Al-4V.[4,20] It is important to note that this method can also be used to form siloxane monolayers that might otherwise be difficult to prepare because of cross-linking or multilayering.[3,25,26] IR analysis of films of **4** and **5** showed $\nu_{\mathrm{CH_2}}$ = 2920, 2860 cm^{-1}, suggesting alkyl chain disorder.[24] The film formed from APTES (**4**) exhibited a free amino group ($\delta_{\mathrm{NH_2}}$ = 1575 cm^{-1}), and **5**, prepared from maleimido adduct **2**, showed ν_{CO} = 1702, 1773 cm^{-1}. Quartz crystal microgravimetry (QCM) measured the surface loadings of **4** and **5** to be 0.27 ± 0.01 and 0.23 ± 0.01 nmol/cm^2, respectively (cross sectional areas 62 and 73 Å^2, consistent with IR determination of alkyl chain disorder). The fact that the loadings of both **4** and **5** are comparable shows that the maleimido functionality is not a limiting factor for surface coverage because of its size relative to that of a simple alkyl chain. To the best of our knowledge, these values are the first reported for direct measurement of surface silanization of Ti; they approximate the calculated content of surface hydroxyl groups on the native oxide of Ti and Ti-6Al-4V (0.34 nmol/cm^2).[27] On the basis of our measured loadings of surface siloxanes **4** and **5** (≈0.25 nmol/cm^2) and assuming that silanes **1** and **2** react with a single, spatially isolated OH as shown in Scheme 1, it can be determined that these silanes consume about 70% of available surface OH groups. In contrast, the surface loading of **7** is 1.00 ± 0.09 nmol/cm^2,[9] corresponding to a cross-sectional area for **7** of ≈16.6 Å^2/molecule. This loading is about four times that of **4** and **5**, and is nearly three times that of the Ti surface OH sites: phosphonate film formation is not limited by surface −OH content.

Table 2. Interfacial Shear Strengths, Uncorrected and Corrected for Surface Loadings

interface precursor	shear strength (MPa)	normalized shear strength (MPa nmol^{-1} cm^{-2})
4	45	58.5
5	34	52.4
7	>52[a]	-

[a] The epoxy, not the interface, fractures.

Shear strengths of interfaces **4**, **5**, and **7** were measured mechanically (Table 2);[22] only the lower limit for the shear strength of **7** could be determined because the epoxy, not the interface, fractured. Differences in interfacial shear strengths of comparably loaded **4** and **5** may be attributed to the respective tail group reactivities with the epoxide. For example, the free amino group of **4** may act as a better nucleophile than the carbonyl oxygen or imido nitrogen groups of **5** and therefore bonds more strongly to the epoxy. In the case of **7**, the high molecular surface density in the interface also provides a closely packed film of nucleophilic OH at the film terminus. All interfacial shear strengths exceed the FDA minimum (20 MP) required for sprayed coatings on surgical implants.[29]

The stabilities of interfaces **4**, **5**, and **7** to hydrolysis at pH 7.5 were measured using fluorophore-modified DANSYL-cysteine derivatives **6** and **9**. Disks of Ti-6Al-4V coated with interfaces of **6** (prepared from both **4** and **5**) and **9** were immersed in water at pH 7.5 for 7 days at room temperature. For the first 5 days, the supernatant was analyzed by fluorescence spectroscopy as a measure of film constituent release. All three interfaces showed an initial loss of multilayer DANSYL-cysteine over the first 6 h of immersion; loss from **9** asymptoted shortly thereafter. In contrast, both siloxane-derived interfaces showed continual loss of DANSYL-cysteine throughout the entire 5 day time period. Surface material stably bound at pH 7.5 after 7 days was then measured by immersing the

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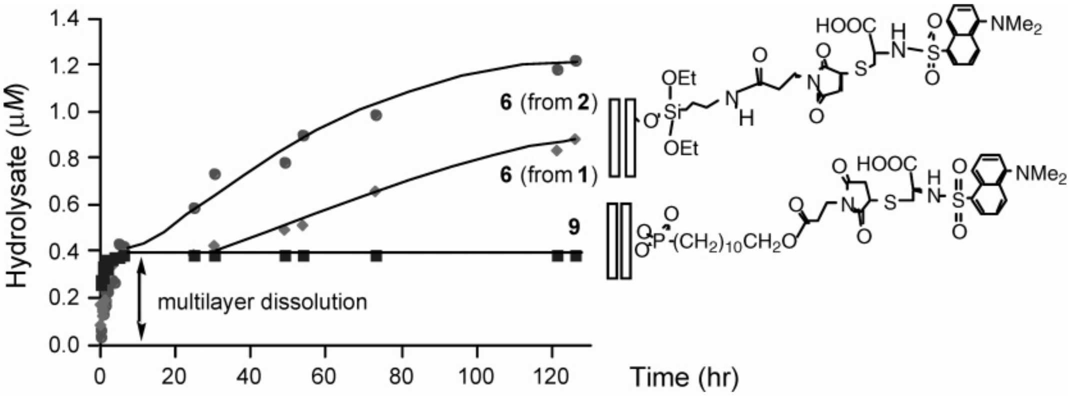

Figure 2. Hydrolytic loss of DANSYL-cysteine from **6** and **9**.

disks in doubly distilled H_2O at pH 12 for 3 h (Figure 2). The difference in stability to hydrolysis of films of **6** formed from the two silane species may be due to the lower surface film density of **6** prepared from **2** than from **1** (see Table 1). Analysis of the supernatants by fluorescence spectroscopy gave the total amount of amino acid hydrolytically removed at pH 12: for **6**, prepared from **4** or **5**, and **9**, these amounts were 0.05 ± 0.03, 0.06 ± 0.03, and 0.35 ± 0.09 nmol/cm^2,[9] respectively. In other words, for siloxanes prepared either from APTES or from its preformed maleimido derivative, virtually all of the initially bound DANSYL-cysteine was cleaved by hydrolysis at the physiological pH of 7.5. In contrast, **9** was stable at pH 7.5; quantification after cleavage at pH 12 showed that the loading of DANSYL-cysteine attached to the surface was nearly identical to its initial loading (the overall yield of **9** from **8** has been measured by QCM to be ca. 35%[9]).

Conclusions

Self-assembled monolayer films of two silane analogues and an alkylphosphonate can be prepared on Ti-6Al-4V using the T-BAG deposition technique.[4] Surface loadings and shear strengths of each film were shown to be sufficient with regard to biomedical device surface coating. However, due to hydrolytic lability of surface Si−O bonds, both silane analogues lost most of the amino acid bound to them after exposure to physiological pH for 7 days, while the amino acid-modified phosphonate film was not similarly degraded. Thus, while silanization may be an effective technique to modify the native oxide coatings of Ti and Ti-6Al-4V from certain perspectives, the inherent instability of siloxane coatings to hydrolysis at physiological pH suggests this technology may be compromised for use in a medical device context.

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