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Modification of an Implant Material

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Introduction:

Infection is a problem for 1 to 13 percent of implants.[8] Titanium is a commonly used implant material due to its corrosion resistance, biocompatibility, and osseointegration.[6] Specifically, titanium is commonly used as an implant material in the mouth and the body, to replace or repair bone tissue in the teeth or skeleton, due to similarities between titanium and bone tissue such as hardness.[1] Titanium therefore, can be coated to prevent bacteria growth and biofilm formation.[1] A biofilm forms when bacteria attaches to the surface of an implant material, and over time forms a colony of bacteria, that is immobile.[8] Biofilms are difficult to treat with systemic antibiotics.

A self-assembled monolayer (SAM) contains a head group and a tail group, and can be used to modify the surface of an implant material. The self-assembled monolayer that was formed in this experiment contained a phosphonic acid head group and a thiol tail group (Figure 1). The head group is where the molecule attaches to the surface of the metal oxide, and the tail group is responsible for the properties of the self-assembled monolayer at the interface.[2] The van der Waals interactions of the alkyl chain aid in film formation. The length of the chain is a factor in this process.[5] Commonly, compounds containing long alkyl chains are used to increase van der Waals interactions, and hence, the stability of the self-assembled monolayer.

Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy can be used to analyze the attachment of a self-assembled monolayer. The instrument contains both an infrared radiation beam and incident beam.[7] When radiation is directed toward the sample on the metal coupon, the radiation is reflected as light off of the sample.[7] This light distributes, and a mirror directs the energy into a detector that changes the energy into a spectrum that can be analyzed.[7]

Hypothesis: Formation of a stable self-assembled monolayer of 12-mercaptododecylphosphonic acid on the surface of titanium will retard biofilm formation and bacteria growth.
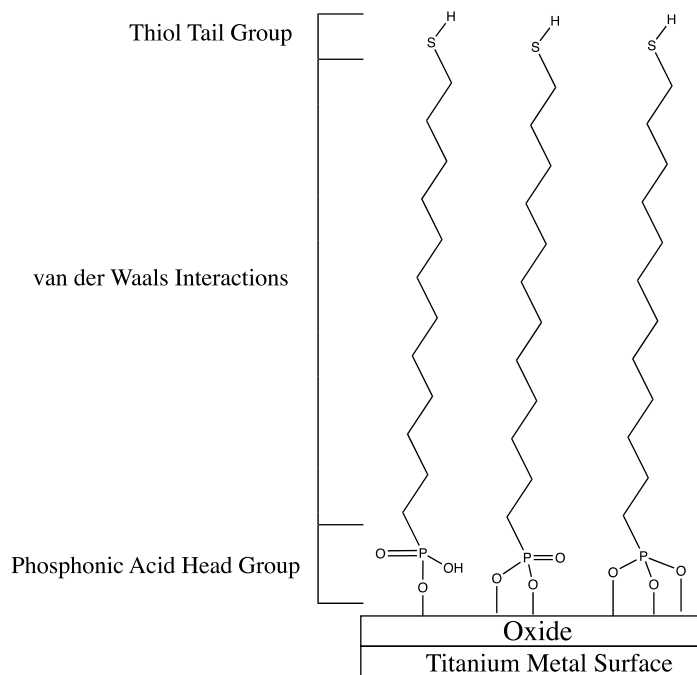
Figure 1: 12-mercaptododecylphosphonic acid SAM on the surface of titanium oxide. The SAM of 12-mercaptododecylphosphonic acid contains a phosphonic acid head group and a thiol tail group. The molecule has a long alkyl chain, in which van der Waals interactions can occur.

Methods:

The titanium oxide surface that was used as the substrate for deposition was 1 centimeter by 1 centimeter square. The titanium metal was previously sanded, and cut into 1 centimeter by 1 centimeter long coupons. The coupons were then sonicated in acetone, to remove any markings that may have been present after the cutting of the metal. Then, the metal was boiled in methanol to remove any residue that may have been present on the surface of the titanium oxide.

A 1 mM solution of 12-mercaptododecylphosphonic acid in tetrahydrofuran (THF) was used for a 1 hour and 2 hour solution deposition. The deposition was conducted by placing coupons of the titanium oxide in a covered beaker containing the 1 mM solution of 12-mercaptododecylphosphonic acid in THF for the desired amount of time. Following the deposition, the samples were dried in the oven at 60ºC for approximately 24 hours. In order to test the stability of the SAMs, a rinse and a sonication in THF were

performed. The samples were rinsed in THF for 15 minutes, then dried in the oven at 60ºC for approximately 24 hours. The samples were then sonicated in THF for 15 minutes, and dried in the oven at 60ºC for approximately 24 hours. The rinse and sonication in THF removed any unbound molecules that may have been on the surface of the titanium oxide. Following the deposition, rinse, and sonication, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy was used to evaluate the attachment, stability, and binding of the self-assembled monolayers to the titanium metal. The instrument was run under N_2 atmosphere, to eliminate possible contaminants of carbon dioxide and water in the air. The spectra produced by DRIFT spectroscopy was analyzed to determine the presence and stability of the self-assembled monolayers on titanium oxide, TiO_2.

In addition to the rinse and sonication in THF, an acid test, base test, and tape test were conducted to determine the stability of the SAMs. The acid test was conducted by placing 3 coupons following the sonication into a beaker containing HCl (1.0 M), and letting the coupons soak for 15 minutes. Then, the coupons were removed from the acid, and rinsed with deionized water, to remove any excess acid. The coupons were then dried in the oven at 60°C for approximately 24 hours, and DRIFT spectroscopy was used to evaluate the SAMs. The base test was conducted by placing 4 coupons following the sonication into a beaker containing NaOH (0.47 M), and letting the coupons soak for 15 minutes. Then, the coupons were removed from the base, and rinsed with deionized water, to remove any excess base. The coupons were then dried in the oven at 60°C for approximately 24 hours, and DRIFT spectroscopy was used to evaluate the SAMs. When analyzing samples after exposure to acid or base, an unmodified TiO_2 coupon was used as the background for DRIFT spectroscopy. The tape test was conducted by placing tape over 3 of the coupons, and peeling the tape away from the coupon. When analyzing samples after the tape test, a TiO_2 coupon upon which tape was placed and removed was used as the background for DRIFT spectroscopy. The background coupon for the tape test did not contain any SAMs, and the deposition, rinse, and sonication were not conducted upon the background coupon. The acid, base, and tape tests

were only conducted following the 2 hour deposition in the 1 mM solution of 12-mercaptododecylphosphonic acid in THF.

Monolayer Characterization:

The SAM was considered ordered if the methylene stretching peaks were $v_{CH2, asym} \leq 2918$ cm^{-1} and $v_{CH2, symm} \leq 2848$ cm^{-1}. The ordered SAM was also considered to have trans-configuration.[3,4] The analysis of the methylene stretching region of the DRIFT spectra that was collected indicated the presence of sub-monolayers if the peak intensity was below 0.30, self-assembled monolayers if the peak intensity was 0.30-0.70, and multilayers if the peak intensity was over 0.70.

The 1 hour deposition produced sub-monolayers, or islands of SAMs. This was determined from the low peak intensity that was observed from the DRIFT spectra. A 2 hour deposition was carried out.
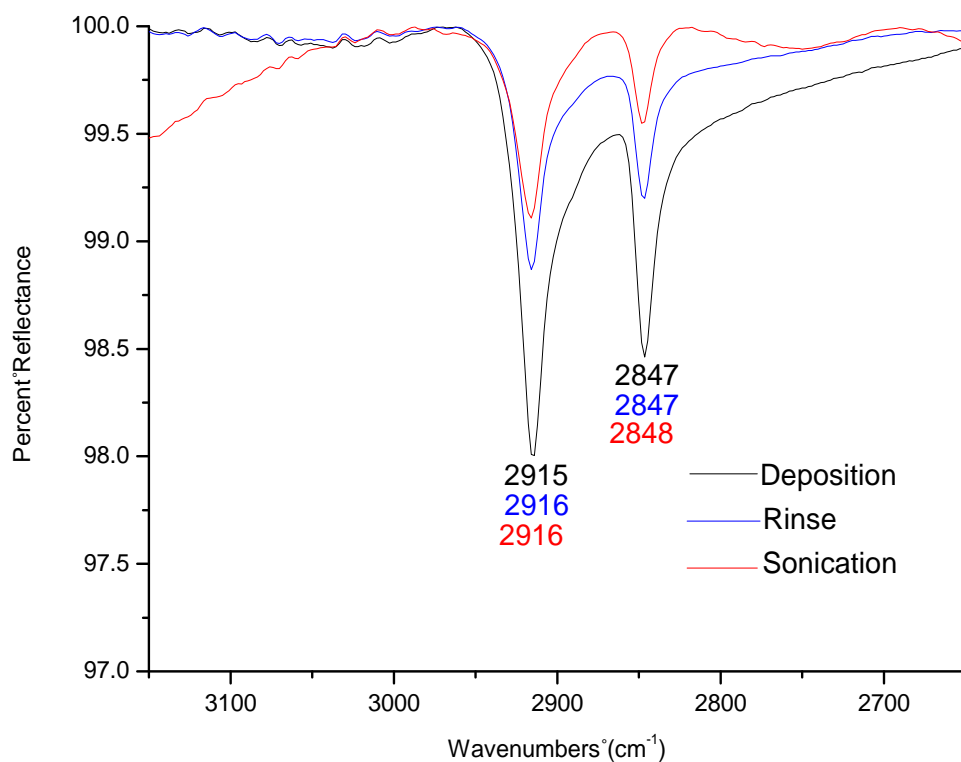

Figure 2: Monolayer attachment, methylene stretching regions of DRIFT spectra after 2 Hr. Deposition.

Sample	Peaks	Peak Intensity after Deposition	Peak Intensity after Rinse	Peak Intensity after Sonication
1	2915, 2847	1.35	0.90	0.85
2	2916, 2848	1.46	0.80	0.90
3	2915, 2848	1.20	1.20	0.72
4	2915, 2845	2.50	1.75	0.57
5	2914, 2845	4.00	0.63	0.80
6	2916, 2847	1.60	1.00	0.75
7	2915, 2847	0.60	0.48	0.20
8	2915, 2845	3.50	1.10	0.70
9	2914, 2845	3.30	0.80	0.55
10	2917, 2848	0.45	1.80	0.35

Table 1: 2 Hr. Deposition Methylene Stretching DRIFT spectra.

Based on the methylene stretching of the DRIFT spectra collected for the 2 hour deposition, the 12-mercaptododecylphosphonic acid SAM on titanium oxide appeared to be ordered and in trans-configuration. It also appeared that after the deposition, multilayers were observed for some of the coupons, and self-assembled monolayers were observed for some of the coupons. Following the rinse and sonication, however, a majority of the coupons of titanium oxide produced self-assembled monolayers, which was indicated by a peak intensity between 0.30 and 0.70. After the sonication, the acid test, base test, and tape test were performed.
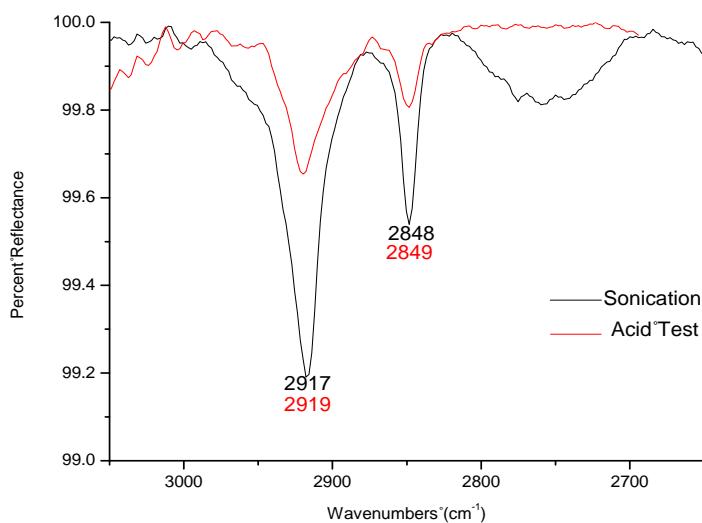

Figure 3: Monolayer attachment, methylene stretching regions of DRIFT spectra following the Acid Test.
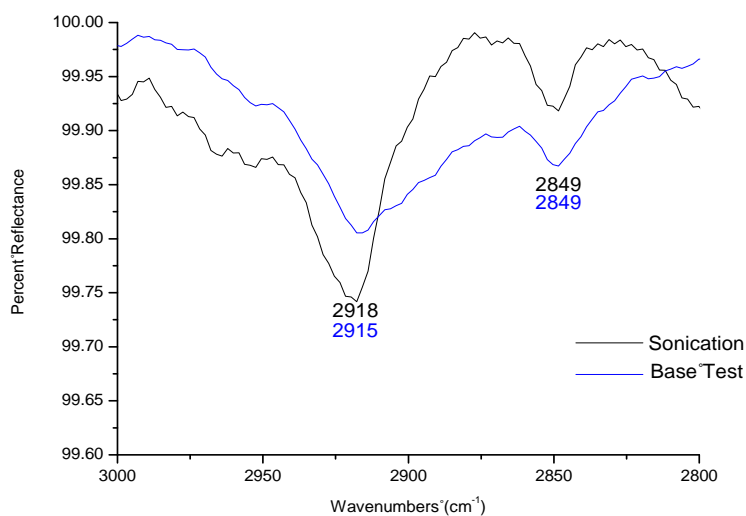
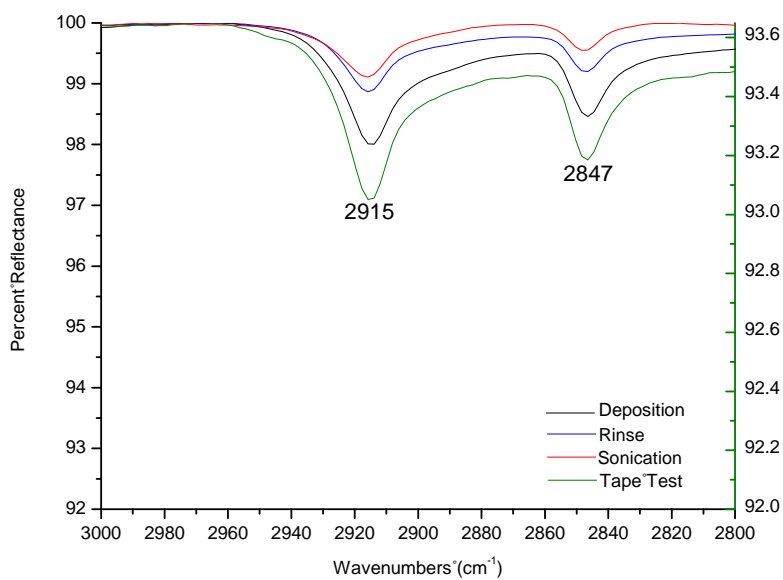**Figure 4**: Monolayer attachment, methylene stretching regions of DRIFT spectra following the Base Test.

Figure 5: Monolayer attachment, methylene stretching regions of DRIFT spectra following the Tape Test.

Sample	Test	Peaks	Peak Intensity
1	Tape	2915, 2847	0.475
2	Tape	2915, 2847	0.575
3	Tape	2914, 2846	0.30
4	Acid	2919, 2849	0.20
5	Acid	2921, 2849	0.26
6	Acid	2919, 2849	0.32
7	Base	2915, 2849	0.10
8	Base	2921, 2856	0.08

| 9 | Base | 2925, 2852 | 0.13 |
| 10 | Base | 2918, 2849 | 0.07 |

Table 2: 2 Hr. Deposition Methylene Stretching DRIFT spectra following Acid, Base, and Tape Tests

It was found that the DRIFT spectrum contained methylene stretching peaks around $v_{CH2, asym} \leq 2918$ cm^{-1} and $v_{CH2, symm} \leq 2848$ cm^{-1} following the acid and tape test, with moderate peak intensities, leading to the conclusion that ordered SAMS were present on the titanium oxide surface. DRIFT spectrum methylene stretching peaks were observed around $v_{CH2, asym} \leq 2918$ cm^{-1} and $v_{CH2, symm} \leq 2848$ cm^{-1}, for the base test with low peak intensities, leading to the conclusion that the SAMs were inconsistently formed on the titanium oxide surface. In other words, the SAMs resided in sub-monolayers, or islands, on the surface of the titanium oxide following the base test. Furthermore, samples 8 and 9 for the base test had peaks that were greater than $v_{CH2, asym} \leq 2918$ cm^{-1} and $v_{CH2, symm} \leq 2848$ cm^{-1}, and were thought to reside in disordered cis-configurations.

Conclusions:

Self-assembled monolayers of 12-mercaptododecylphosphonic acid were formed on the surface of titanium oxide, and remained after the acid test and base test. SAMs were weakly present following the base test. The binding modes were unable to be observed. Future work will be to use the SAMs of 12-mercaptododecylphosphonic acid as anchors for the immobilization of bioactive molecules due to the stability of the thiol tail group.

Acknowledgements:

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