Surface Modification of Titanium by Atomic Transfer Radical Polymerization (ATRP)

Tyler G. Libey,1 Gilad Zorn,2 David G. Castner2,3
1School of Biological & Health Systems Engineering, Arizona State University, Tempe, Arizona 85287
2Department of Chemical Engineering, University of Washington, Seattle, Washington 98195
3Department of Bioengineering, University of Washington, Seattle, Washington 98195

Abstract: Titanium is commonly used as an orthopedic biomaterial due to its unique mechanical properties and good corrosion resistance. However, after implantation into the body, titanium does not always integrate completely with bone. Its performance can be improved by surface modification with an osteointegrative, bioactive coating such as poly(sodium styrene sulfonate) (pNaSS). Thin films of NaSS were grafted onto rough polished Ti foil as well as smooth, thin Ti layers (~50nm thickness) evaporated onto Si wafers. These substrates were first modified with a chloroform solution of methacryloxypropyltrimethoxysilane (MPS) and then immersed into different NaSS solutions. The grafted surfaces were characterized by a colorimetric assay and X-ray photoelectron spectroscopy. The atomic transfer radical polymerization (ATRP) reaction was catalyzed by either heating the solution to 90°C or by using CuBr at room temperature; the latter resulted Cu contamination in the grafted layer. The reaction kinetics were studied and 15 hrs was found to be an optimum duration for grafting the NaSS layers. It was also found that unlike grafting under nitrogen flow, grafting under vacuum produces reproducible NaSS films.

1. INTRODUCTION

The demand for hard tissue replacements, such as artificial hip joints and dental implants is ongoing [9]. These hard tissue implants require a material that combines mechanical strength and biocompatibility. Metals are commonly used as orthopedic and dental biomaterials due to their comparable specific strength to bone [8]. Among the metals, titanium and its alloys are considered to have high strength, relatively low Young’s-modulus, and high corrosion resistance while exhibiting minimal allergic reactions. However, titanium and its alloys are considered bioinert and typically do not allow direct bonding with the bone tissue or promotion of osteogenesis [8].

The ability for the implant to integrate with the bone (osteointegration) is crucial in preventing implant failure [6]. Osteointegration is possible through biomechanical bonding and biochemical bonding [1]. Biomechanical bonding describes the affinity for bone growth into the implant and causes mechanical stability. This interaction, however, can take up to 1-2 years to reach maximum stability. Biochemical bonding between the implant and bone, on the other hand, represents a more immediate stabilizing reaction [1], as well as additional osteogenesis promotion [8]. Surface treatments to titanium implants have been shown to increase protein adsorption and cell attachment, thereby increasing the affinity for a surface to bond [5].

Many polymers have demonstrated relatively high bonding characteristics and flexibility. For example poly(sodium styrene sulfonate) (pNaSS) has been shown to directly enhance osteoblast functionality [7]. Thus, a method for grafting pNaSS onto titanium should increase the overall effectiveness of metallic implants. While pNaSS cannot be grafted directly onto titanium, an intermediate silane layer can be used as a cross linker between the solid surface and the grafted layer [7].

In this work, bioactive NaSS was grafted onto surfaces of titanium through atomic transfer radical polymerization (ATRP). ATRP can be used to form a dense polymer brush on the surface of materials [3]. Methacryloxypropyltrimethoxysilane (MPS) was used as an intermediate silane layer. The grafting procedure was optimized through examination of a combination of a toluidine blue colorimetric assay [2] and X-ray photoelectron spectroscopy (XPS). Through the process of silanization and ATRP, a robust coating was bound to the titanium substrate.

2. MATERIALS AND METHODS

2.1 Surface Preparation
Two types of titanium surfaces were prepared. 1cm² squares were cut from titanium foil (Sigma), then were polished sequentially using 3µm, 1µm, .25µm, and .05µm diamond glycol suspension polish. The polished samples (Tifoil) were sonicated in dichloromethane, acetone and ethanol (twice at 20 min each) and then stored in air-tight microchip wafer containers until further use. Smooth titanium samples (Ti₇) were prepared by evaporation of thin Ti layers (~50nm) onto 1cm² silicon wafers.

2.2 Silanization

The samples were sonicated in chloroform (CHCl₃) for 15 min, rinsed in CHCl₃ and soaked in a solution of 19mL CHCl₃ and 1mL methacryloxypropyl-trimethoxysilane (Gelest) (5%v/v) for 1 hr. The samples were gently rinsed in CHCl₃ and then placed in a 140°C oven for 4 hr. The samples were finally sonicated in CHCl₃ for 30 min, allowed to air dry, and stored in air-tight microchip wafer containers.

2.3 pNaSS Grafting through ATRP

1.4g of 4-vinylbenzensulfonic acid sodium salt (0.7M NaSS monomer), 15mg of 2,2-azobisisobutyronitrile (9mM, AIBN) initiator, were dissolved in 10mL of dimethyl sulfoxide (DMSO) (Sigma). The silanized titanium substrates were placed in the solution and the solution was degassed for 2 hr prior ATRP.

Catalyst: During the reaction the solution was subjected to one of two types of catalysts: 1) heating the solution to 90°C [2] or 2) addition of 25mg of 98% pure copper bromide (Cu(I)Br) and 60mg of bipyridine to the reaction mixture [4]. The chemically catalyzed polymerization occurred at room temperature.

Atmospheric Condition: ATRP was performed either under nitrogen flow [7] or under vacuum.

Grafting Kinetics: The extent of reaction was examined at 5, 10,15, and 24 hr.

2.4 Surface Characterization

Atomic Force Microscopy (AFM): AFM measurements were preformed with a Dimension 3100 AFM (Veeco Metrology Inc., Santa Barbara, CA). The AFM was equipped with a 315 kHz, 42 N/m PointProbe Plus silicon tip (Nanosensors, Neuchatel, Switzerland) and operated in the intermittent contact mode in air. AFM measurements were used to characterize and compare the surface roughness of both samples (Tifoil and Ti₇).

Colorimetric Assay: The colorimetric assay procedure was adopted from Ciobanu et al [2]. 7.5g of toludine blue was added to 50mL DI-H₂O. A buffer solution of 0.2mL of 2-amino-2-methyl propanol was then added. Each polymerized sample was then placed in an individual well of a 6-well plate with 6mL of the solution and incubated at 30°C for 6 hr. The samples were then soaked in a 5x10⁻⁴M NaOH solution for 30 min. Each sample was then placed in a new well with 5mL of acetic acid (50%aq) for 24 hr. The remaining de-complexed solution was then analyzed using a UV/visible plate reader and the polymer surface density was calculated as follows [2]:

\[ \text{g grafted polymer/cm}^² \text{ Ti} = \frac{\text{CVM}}{2} \]

where \( C \) = molar concentration of the monomer in the de-complexed solution, \( V \) = acetic acid volume (0.005L), and \( M \) = molar weight of the monomer (206g/mol). Concentration was calculated using the Beer-Lambert law:

\[ C = \frac{A}{\varepsilon l} \]

where \( A \) = measured absorbance, \( \varepsilon \) = characteristic absorbance of toludine blue (50,000 L/(Mol*cm)), and \( l \) = path length of the sample.

X-ray Photoelectron Spectroscopy (XPS): XPS data was acquired with a Surface Science Instruments S-probe spectrometer. This instrument has a monochromatized Al Kα X-ray source, hemispherical analyzer, multichannel detector and low-energy electron flood gun for charge neutralization. The X-ray spot size used for these experiments was approximately 800 µm x 800 µm. Pressure in the analytical chamber during spectral acquisition was less than 5 x 10⁻⁹ torr. Spectra used to determine surface elemental compositions were acquired at an analyzer pass energy of 150 eV. The high-resolution spectra were acquired at analyzer pass energy of 50 eV.
Table 1. XPS analysis of bare titanium substrates (Ti_foil and Ti_Si) yielded similar amounts of Ti, C, and O characteristic of a titanium oxide surface covered with adventitious hydrocarbons. XPS of the MPS treated substrates showed the presence of silicon, indicating a silane layer was formed. pNaSS grafting yielded a surface composition containing sulfur and sodium which are both present in the pNaSS polymer. Additionally, the two types of substrates, Ti_foil and Ti_Si, exhibited similar at% compositions throughout each step of the surface modification.

<table>
<thead>
<tr>
<th></th>
<th>Ti_foil</th>
<th>Ti_Si</th>
<th>Ti_foil + MPS</th>
<th>Ti_Si + MPS</th>
<th>Ti_foil + MPS + pNaSS</th>
<th>Ti_Si + MPS + pNaSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Ti 2p</td>
<td>14.0±1.1</td>
<td>12.7±0.3</td>
<td>13.3±0.7</td>
<td>13.2±0.5</td>
<td>7.22±0.4</td>
<td>7.1±1.3</td>
</tr>
<tr>
<td>%O 1s</td>
<td>44.4±1.3</td>
<td>50.4±0.4</td>
<td>51.1±0.4</td>
<td>50.7±2.0</td>
<td>42.2±0.6</td>
<td>37.5±5.4</td>
</tr>
<tr>
<td>%C 1s</td>
<td>41.2±0.3</td>
<td>36.9±0.1</td>
<td>32.1±0.3</td>
<td>31.3±1.6</td>
<td>46.2±0.2</td>
<td>50.7±6.5</td>
</tr>
<tr>
<td>%Si 2p</td>
<td>-</td>
<td>-</td>
<td>3.6±0.3</td>
<td>4.9±0.3</td>
<td>1.9±0.1</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>%S 2p</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.9±0.1</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Na 1s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.41±0.1</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

The take-off angle (the angle between the sample normal and the axis of the analyzer lens) was 55° for all XPS experiments. Three spots on two replicates were analyzed for each sample type.

The ESCA Analysis A program was used to determine peak areas and calculate elemental compositions. The binding energy (BE) scale was calibrated by assigning the hydrocarbon peak in C1s high-resolution spectra to a BE of 285.0 eV.

3. RESULTS

3.1 Bare Titanium

The Ti_foil substrate was significantly rougher than the Ti_Si substrate (Figure 1). Ti_foil exhibited a roughness coefficient of 79nm, while Ti_Si had a roughness coefficient of 1.1nm. The surface composition of each type of substrate, however, was fairly similar (Table 1). Both substrates exhibited an XPS surface composition containing titanium, oxygen, and carbon.

3.2 Silane Composition

Silanization was successful on both the Ti_foil and the Ti_Si (Table 1). XPS data showed that the silicon concentration was 3-5 atomic % after silanization.

3.3 Grafting Kinetics

It was observed that the reaction times directly influenced the amount of the NaSS monomer polymerized onto the surface. It was expected that longer times will increase the surface density of the grafted polymer. However, based on toluidine blue colorimetric assay, the grafting levels appeared to plateau at approximately 300ng/cm² (Figure 2). A polymerization time of 15 hr yielded 240ng/cm². This represents a grafting increase of 62% from 10 hr and 140% from 5 hr. From 15 hr to 24 hr, however, only a 11% increase in grafting was observed. Similar increases were observed across all combinations of variables.
3.4 Atmospheric, Catalyst and Substrate Conditions

Samples catalyzed with the Cu(I)Br catalyst yielded polymer layers contaminated with Cu (Table 2). In addition it was found that polymerization under flowing nitrogen yielded non-uniform polymer layers with high standard deviations of the C:Ti atomic ratio compared to polymerization under vacuum (Table 3). Polymerization under vacuum for 15 hr at 90°C yielded a C/Ti ratio of 6.9±1.2. Both substrates yielded similar surface compositions under these specific conditions (Table 1).

<table>
<thead>
<tr>
<th>Nitrogen Flow</th>
<th>Vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>at % Cu</td>
<td></td>
</tr>
<tr>
<td>0.2 ± 0.4</td>
<td>2.2±2.5</td>
</tr>
</tbody>
</table>

*Table 2. The XPS Cu concentrations after the 15 hr, Cu(I)Br catalyzed reactions under both flowing nitrogen flow and vacuum.*

<table>
<thead>
<tr>
<th>C:Ti atom ratio</th>
<th>Nitrogen Flow</th>
<th>Vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.4±16.7</td>
<td></td>
<td>6.9±1.2</td>
</tr>
</tbody>
</table>

*Table 3. The XPS C/Ti atomic ratios after the 15 hr, heat catalyzed reactions under flowing nitrogen and vacuum. The C/Ti atomic ratio was higher under flowing nitrogen, but also had a significantly higher standard deviation.*

4. CONCLUSION

A few key observations can be made from these results. First, it has been shown that titanium evaporated onto silicon is a convenient smooth model titanium surface for grafting studies. In addition, the conditions for grafting NaSS onto titanium were optimized. A copper catalyst cannot be used since it introduces copper into the polymer layer. Flowing nitrogen during reaction resulting in formation of a non-uniform polymer layer. It was shown that as the grafting time increased from 0 to 15 hr, the amount of NaSS grafted to the surface increased. From all experimental conditions examined, an optimized procedure for grafting NaSS onto Ti surfaces under vacuum at 90°C for 15 hr was established.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the University of Washington Engineered Biomaterials program, the National Science Foundation, and the National Institutes of Health. (Grant #0647918, EEC-9529161, and EB-002027)

REFERENCES


