Effect of Storage Conditions on Ti-6Al-4V Surface Wettability

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The relationship between storage conditions and wettability of annealed micro-rough samples was investigated in this study. Micro-roughness (2.45 ± 0.43 µm) was obtained through sandblasting and acid-etching in sulfuric acid and hydrogen peroxide. Non-annealed samples (control) and samples annealed at 400 °C and 600 °C were used. In order to investigate the influence of storage conditions on the surface wettability, Ti-6Al-4V disks were 1) wrapped in Kimwipe inside a plastic bag, 2) stored in petri-dishes containing 20 mL deionized water (DI-water) at room temperature, and 3) stored in petri-dishes containing 20 mL cold DI-water (8 ± 2 °C). Surface characterization was conducted using water contact angle (WCA) measurements, white light interferometry (WLI), and fourier transform infrared (FTIR) spectroscopy. Disks immersed in DI-water maintained higher wettability when compared to Kimwipe groups over the course of 34 days regardless of the storage temperature. In addition, samples annealed at 600 °C showed superior wettability due to the rutile nature of the oxide film.

Introduction

Titanium based materials are extensively used for biomedical applications such as dentistry, orthopedics and cardiovascular surgery. In 1969, Branemark observed that titanium had good adhesion when in contact with rabbit bone and was highly biocompatible.1 Since then, numerous studies have been conducted confirming titanium and its alloys as suitable choices for such applications. As reported in 2010, 1000 tons of titanium is used for biomedical purposes worldwide every year on average.2 Biomaterials, materials used in biomedical applications, have to exhibit a superior combination of several characteristics: adequate mechanical properties, high corrosion and wear resistance, high osseointegration and, most importantly, high biocompatibility.3 Inadequate mechanical properties such as low fracture toughness or low fatigue strength can cause implants to fail. Properties such as elasticity modulus can induce mechanical failure through stress shielding effect.4 The stress cannot be fully transferred through the biomaterial and is reflected, causing implant loosening. Titanium and its alloys are found to present better combination of such properties when compared to other biomaterials such as Co-Cr and Stainless Steel.

Corrosion and wear can accelerate the process of mechanical failure. In addition, metal ions released due to corrosion and wear from Co-Cr implants were found to induce the formation of cancerous tumors.2 Titanium is known to be inert in all body fluids, therefore corrosion resistant.2 However, wear and fretting can damage the passive film and lead to transient corrosion. All the oxidative reactions occur during the repassivation time, gap between the damage and the reformation of the film. Compared to stainless steel 316L, Co-28Cr-6Mo and Zr-2.5Nb, Ti-6Al-4V had a faster repassivation time.2

Poor osseointegration, the connection between the implant and the living bone, can result in implant loosening and failure.3 Better cell attachment, proliferation and differentiation result in superior osseointegration.3

Hydrophilicity is an indicator of cell attachment, since the more hydrophilic a surface is the more proteins attach over the water molecules which will lead to more cell adhesion on the implant.

One way to improve the hydrophilicity of a surface has been demonstrated by Park et al. and Watanabe et al.4,5 They observed significant changes in the water contact angle related to sterilization methods such as Oxygen Plasma, Ultraviolet and Gamma Irradiation.4,5 Furthermore, they mentioned that the surface wettability decreases over time due to poor storage methods.4 In this respect, improvement in storage methods could prevent potentially good biomaterials to be classified erroneously as bad by further cell culture tests.

The gap of time between samples preparation and cell culture tests may be a factor that is characterizing some biomaterials as not suitable. At least one company stores their implants in liquid solutions to keep them hydrophilic for an extended shelf life.6 Preliminary water contact angle measurements conducted in our lab suggested that storing Ti-6Al-4V samples in water, rather than in air, might keep the hydrophilicity obtained by
surface modification treatments longer when compared to those stored in air. However, a decrease of wettability over time was still observed. As reported by Linsebigler et al., increase in temperature correlates with water desorption and loss of hydroxyl groups at ultra-high vacuum condition. The presence of these hydroxyl groups is found to be responsible for the increase in wettability of the TiO$_2$ passive layer. This study evaluates the effect of storage conditions on Ti-6Al-4V samples surface wettability.

**Experimental Description**

**Sample Preparation and Storage**

Ti-6Al-4V discs (15 mm in diameter and 1 mm thick) were obtained from McMaster-Carr (Part Number 89055K57). To obtain micro-rough surfaces, the Ti-6Al-4V discs were blasted with 50µm alumina grit particles (Alumina (Al$_2$O$_3$) powder, Trinity Tool Company, Fraser MI) at a pressure of 517 kPa and acid-etched in a solution of 1:1 ratio of 100% sulfuric acid to 30% hydrogen peroxide for 2 hours, washed with DI-water of 17.7 MΩ-cm resistivity (Barnstead NANOPure, Infinity UltraPure Water System®). Micro-rough surface samples were temporarily stored submerged in DI-water in a glass dish.

As detailed in Table I, the disks were divided into 9 groups, which were non-annealed and annealed at 400°C and 600°C in a furnace (Lindberg furnace, S #54032) for three hours in open air and stored in petri-dishes (Advantec, MFS, Inc.) submerged in 20 mL of room temperature (RT) or cold DI-water. Samples in cold water were stored in a refrigerator (Haiser) with average temperature of 8 ± 2°C. Before annealing the disks were cleaned with sonication for 1 hour in 99.8% methanol (Sigma Aldrich) using an Ultrasonic Wafer Cleaning System (Branson) and washed for 30 seconds with DI-water of 17.7 MΩ-cm resistivity (Barnstead NANOPure, Infinity UltraPure Water System®). Micro-rough surface samples were stored wrapped in Kimwipe and in petri-dishes either at room temperature (25°C) or cold (8 ± 2°C).

<table>
<thead>
<tr>
<th>Group</th>
<th>Annealing</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not Annealed</td>
<td>RT DI-Water</td>
</tr>
<tr>
<td>2</td>
<td>Annealed at 400°C</td>
<td>Cold DI-Water</td>
</tr>
<tr>
<td>3</td>
<td>Annealed at 600°C</td>
<td>Cold DI-Water</td>
</tr>
<tr>
<td>4</td>
<td>Annealed at 400°C</td>
<td>Kimwipe</td>
</tr>
<tr>
<td>5</td>
<td>Annealed at 600°C</td>
<td>RT DI-Water</td>
</tr>
<tr>
<td>6</td>
<td>Annealed at 400°C</td>
<td>Cold DI-Water</td>
</tr>
<tr>
<td>7</td>
<td>Annealed at 600°C</td>
<td>Kimwipe</td>
</tr>
<tr>
<td>8</td>
<td>Annealed at 600°C</td>
<td>RT DI-Water</td>
</tr>
<tr>
<td>9</td>
<td>Annealed at 600°C</td>
<td>Cold DI-Water</td>
</tr>
</tbody>
</table>

**Surface Characterization**

**Roughness**

A white light interferometric (WLI ) microscope (NewView 6300, Zygo Corporation, Middlefield, Connecticut, USA) was used to measure surface roughness and generate 3-dimensional (3D) profiles of the surface. This is an optical microscope which gathers light from the test area (0.177 mm x 0.133 mm) of the sample. The objective lens divides light into 2 paths, one that shiines onto the surface of the test material, and another into an internal reference surface. Surface irregularities on the test material cause the measurement wave-front to travel different distances than the reference wave-front, which cause interference bands when they are recombined. This interference is read by a photo-detector, which translates the interference wave-front into a 3D image, representing the surface topography.

**Chemistry**

Surface composition was studied using absorbance fourier transform infrared (FTIR) spectroscopy (Nicolet, S#ADU9700221). A non-annealed sample was used as a control/background. Prior to conducting FTIR analysis, each sample was left inside the FTIR chamber during purging for one hour in order to optimize signal to noise ratio. FTIR spectrum was obtained with the resolution of 1 cm$^{-1}$ and 512 scans over the range of 4000-400 cm$^{-1}$.

**Wettability**

Prior to water contact angle (WCA) measurement, fifteen seconds of nitrogen gas (Zero Grade, Airgas) spray was used to remove any residue deposited on the surfaces of samples stored in Kimwipe and to dry samples stored in DI-water. Using a micro-syringe (Hamilton), a drop of 5 µL of DI-water was gently placed on the center of the sample. The WCA was then measured using a Ram-Hart NRL CA Goniometer (M#100-0, S#2067) on both sides of the drop. The value computed for each disk is the average of these two.

Preliminary work in the lab showed drastic change in WCA over the course of one day after heat treatment. To observe short term and long term changes, WCA measurements were conducted after 0, 3, 6 and 12 hours and 1, 2, 6, 9, 13, 6 and 20 days. Samples stored in cold DI-water were warmed up to room temperature on Day 20 and subsequent measurements were performed after...
3, 6, 12 and 24 hours and 3 and 7 days. After these sets of measurements, on day 27, all samples were immersed in DI-water and warmed up to 37°C, average body temperature, using a heating plate (Corning Glass Works) wrapped in a flexible heating tape (OMEGAFLUX). Temperature was monitored using a digital thermometer with a TYPE K thermocouple (Omega Engineering Inc., HH-25KC, S# T-116666).

After a WCA measurement was taken, each disk was dried for fifteen seconds with nitrogen gas and restored to respective storage condition. For WCA measurements of sample groups stored in cold water, samples were transferred to another petri-dish with 20 mL of DI-water for 10 minutes to warm up to room temperature. They were not cold while the measurement was performed.

Arithmetic average of the five measurements in each group is used to represent the group tendency. And population standard deviation was used as the error. The distribution of the data was assumed to be normal. Two disks (24 and 63) were identified as outliers using Chauvenets Criterion and removed from data shown hereafter.

**Results and Discussion**

**Zygo**

As shown in Table II, micro-roughness achieved by sandblasting and acid-etching is given in root-mean-square (RMS) and average roughness (RA). The different annealing temperatures did not change the average roughness significantly.

<table>
<thead>
<tr>
<th>Roughness (µ m)</th>
<th>Not Annealed</th>
<th>400°C</th>
<th>600°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMS</td>
<td>2.27 ± 0.27</td>
<td>2.57 ± 0.47</td>
<td>2.51 ± 0.56</td>
</tr>
<tr>
<td>RA</td>
<td>1.76 ± 0.16</td>
<td>2.02 ± 0.41</td>
<td>1.97 ± 0.48</td>
</tr>
</tbody>
</table>

**FTIR**

Figure 1 shows the FTIR spectrum of a non-annealed sample. Due to the FTIR apparatus requirement of a background sample for comparison, the non-annealed sample is also the background. With the exception of the presence of water and carbon dioxide in the FTIR system that appear as several peaks around 667, 1600 and 2400 cm⁻¹, respectively, there are no other significant peaks. Notable peaks are present in the spectra of annealed samples at 400 and 600°C (Figures 2 and 4). Peak deconvolution points to the presence of Ti-O bonds and anatase on the samples annealed at 400°C at 550, 590, 690, and 870 cm⁻¹, respectively. In addition, signatures related to vanadium (760 cm⁻¹ and 715 cm⁻¹) and aluminum oxides (960 cm⁻¹) and rutile (830 cm⁻¹) are shown by the peak deconvolution for spectrum of the samples annealed at 600°C.

This suggests that annealing promotes the formation of crystalline TiO₂ structures and higher annealing temperatures result in reactions between oxygen and other alloy elements in addition to titanium. Further studies need to be carried out to confirm the formation of vanadium and aluminum oxides for higher annealing temperatures and to better establish their ratio compared to titanium oxide as well as compare the amount of anatase and rutile titanium oxides formed on the surface.

**FIG. 1:** Control Sample (Not Annealed) FTIR Spectrum. Background noise from carbon dioxide (667 and 2300-2400 cm⁻¹) and water (1200-2000 and 3000-3800 cm⁻¹) in the air and on the sample surface is seen.

**FIG. 2:** Sample Annealed at 400°C FTIR Spectrum and Peak Detail. Background noise from carbon dioxide (667 and 2300-2400 cm⁻¹) and water (1200-2000 and 3000-3800 cm⁻¹) in the air and on the sample surface is seen. Peak deconvolution using XPSPEAK shows growth of anatase on the surface (870 cm⁻¹).
FIG. 3: Sample Annealed at 600°C FTIR Spectrum and Peak Detail. Background noise from carbon dioxide (667 and 2300-2400 cm$^{-1}$) and water (1200-2000 and 3000-3800 cm$^{-1}$) in the air and on the sample surface is seen.\textsuperscript{9–11} Peak deconvolution using XPSPEAK shows growth of anatase (550 cm$^{-1}$, 590 cm$^{-1}$, 690 cm$^{-1}$ and 870 cm$^{-1}$) and rutile (830 cm$^{-1}$).\textsuperscript{15} Also, signatures of vanadium oxide (760 cm$^{-1}$ and 715 cm$^{-1}$)\textsuperscript{15} and aluminum oxide (960 cm$^{-1}$)\textsuperscript{14} are seen.

FIG. 4: Sample Annealed at 600°C FTIR Spectrum and Peak Detail. Background noise from carbon dioxide (667 and 2300-2400 cm$^{-1}$) and water (1200-2000 and 3000-3800 cm$^{-1}$) in the air and on the sample surface is seen.\textsuperscript{9–11} Peak deconvolution using XPSPEAK shows aluminum oxide signatures (447 cm$^{-1}$ and 485 cm$^{-1}$).

Water Contact Angle

As seen in Figures 5-7, samples wrapped in Kimwipe for all annealing conditions became very hydrophobic in less than a day, WCA reaching as high as 120°C. Comparatively, all the samples stored in DI-water kept low water contact angles throughout the entire study regardless of the storage temperature. Furthermore, an increase in wettability was observed on the samples wrapped in Kimwipe after they were immersed in DI-water on day 27.

In addition, samples annealed at 600°C and wrapped in Kimwipe (group 7) took more time to reach the level of hydrophobicity observed in groups 1 and 4, stored in the same condition but annealed at 400°C and non-annealed. Samples in group 7 water contact angle only passed 100° on day 6, whereas group 1 reached this level on day 1 and group 4 on day 2. Also, the increase in wettability in
FIG. 7: Water Contact Angle Evolution of Samples Annealed at 600°C. Water contact angle increases drastically for samples wrapped in Kimwipe (group 7). In the same group, increase in wettability is observed once the samples are immersed in warm DI-water. Groups 8 and 9 maintained low water contact angle.

This difference may be due to the difference in surface chemistry. As discussed earlier, samples annealed at 600°C show absorbance signatures related to vanadium and aluminum oxides and rutile titanium oxide. Further studies to understand what exactly causes increase in wettability related to increase in annealing temperature can be carried out.

Summary

Micro-roughness (2.45 ± 0.43 µm) was achieved by sequential sandblasting and acid-etching. Samples were annealed at 400°C and 600°C. FTIR confirmed presence of anatase and rutile titanium oxides for 400°C and 600°C, respectively. Interestingly, FTIR peak deconvolution showed signatures of vanadium and aluminum oxides on samples annealed at 600°C. Storing samples in DI-water was found to maintain high wettability regardless of storage temperature. In addition, samples annealed at 600°C had better wettability than those annealed at 600°C and non-annealed samples due to the rutile nature of the oxide film, which is known to dissociate water at a higher rate than anatase.

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