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An XPS study on the attachment of triethoxsilylbutyraldehyde to two titanium surfaces as a way to bond chitosan

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Abstract

A bioactive coating has the ability to create a strong interface between bone tissue and implant. Chitosan, a biopolymer derived from the exoskeletons of shellfish, exhibits many bioactive properties that make it an ideal material for use as a coating such as antibacterial, biodegradable, non-toxic, and the ability to attract and promote bone cell growth and organized bone formation. A previous study reported on the bonding of chitosan to a titanium surface using a three-step process. In the current study, 86.4% de-acetylated chitosan coatings were bound to implant quality titanium in a two-step process that involved the deposition of triethoxsilylbutyraldehyde (TESBA) in toluene, followed by a reaction between the aldehyde of TESBA with chitosan. The chitosan coatings were examined on two different metal treatments to determine if any major differences in the ability of titanium to bind chitosan could be detected. The surface of the titanium metal and the individual reaction steps were examined using X-ray photoelectron spectroscopy (XPS). Following the deposition of TESBA, significant changes were seen in the amounts of oxygen, silicon, carbon, and titanium present on the titanium surface, which were consistent with the anticipated reaction steps. It was demonstrated that more TESBA was bound to the piranha-treated titanium surface as compared to the passivated titanium surface. The two different silane molecules, aminopropyltriethoxysilane (APTES) and TESBA, did not affect the chemistry of the resultant chitosan films. XPS showed that both the formation of unwanted polysiloxanes and the removal of the reactive terminal groups were prevented by using toluene as the carrier solvent to bond TESBA to the titanium surfaces, instead of an aqueous solvent. Qualitatively, the chitosan films demonstrated improved adhesion after using toluene, as the films remained attached to the titanium surface even when placed under the ultra-high vacuum necessary for XPS, unlike the chitosan films deposited using an aqueous solvent, which were removed when exposed to the ultra-high vacuum environment of XPS.

Keywords: Biopolymer; Chitosan; XPS; Biomedical coatings; Triethoxsilylbutyraldehyde

1. Introduction

The ability of surrounding bone tissue to incorporate an implant, also called osseointegration, is a major issue with orthopaedic and dental/craniofacial implants. One way to improve osseointegration is to bond bioactive coatings to the implant surface. Several different bioactive materials are currently being investigated, such as enzymes and proteins [1–3], hydroxyapatite and calcium phosphate [4–8], and bioactive glass [9], which allow the attachment and growth of bone cells into the implant, improving the implant's stability [10]. Several of the bioactive coatings currently being investigated, including hydroxyapatite, calcium phosphate, and bioactive glass, are considered ceramics or glass–ceramics [5,10]. During surgery to place the implant into the human body, stresses are placed upon the coatings that the brittle nature of ceramics and glass–ceramics cannot withstand, leading to cracking and flaking of the coatings [11]. Osseointegration of the implant is then reduced or prevented due to the removal of the coating, which allows fibroblast growth and prevents the production of ordered bone tissue [12].

Bioactive polymers may overcome issues regarding the brittle ceramic material coatings for bone implants. Chitosan is one such bioactive polymer that has shown promise as an

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implantable material [13]. Chitin is found in the exoskeletons of shellfish, arthropods, and the cell walls of fungi [14,15]. Through chemical treatment, chitin can be de-acetylated, forming chitosan [15]. This de-acetylation of chitin into chitosan produces more amine groups in the chitosan chain, which then become protonated in solution [12]. The positively charged chitosan chains attract proteins and cells and promote cell adhesion [12,16]. Chitosan also prevents the growth of fibroblasts, allowing for the growth and replication of osteoblasts that produce orderly bone tissue [12,17]. Because chitin is produced biologically, the by-products of the degradation of chitosan are part of normal cellular metabolism, indicating that chitosan is both biodegradable and non-toxic, with an LD50 greater than 16 g/kg [15,16,18]. Chitosan also possesses bactericidal properties, with the ability to kill *Staphylococcus epidermis*, *Staphylococcus aureous*, and members of the yeast family, Candida, and bacterialstatic properties with the ability to prevent the replication of *Pseudomonas aeruginosa* [14,19].

Chitosan originally was investigated as bone filler for holes produced by wisdom teeth extraction [20–23] and for wound dressings [24]. Very little has been done, however, to investigate the bonding of chitosan to implant quality metals, despite the ability of chitosan to produce ordered bone tissue. The few tests that have been performed on bonding chitosan to a substrate have been performed on plastic or glass dishes [25–27]. An understanding of the surface chemistry needed to bond chitosan to a material has not been developed. The research efforts that do involve coating a substrate with chitosan did not examine the surface chemistry involved in the bonding process, but instead focused on building a film on top of the substrate. The most fundamental method to attach chitosan to a substrate is evaporation, where a chitosan solution is poured over the substrate and the solvent is allowed to evaporate [28]. Chitosan films can also be created by reacting the substrate with a silane molecule, followed by a linker molecule, and finally through evaporation of the chitosan solution [16,29]. The silanation reaction produced an increase in the bond strength of the chitosan film to the substrate (1.5–1.8 MPa) as compared to the simple evaporation method (0.5 MPa) [16]. The reported bond strengths of hydroxyapatite coatings (6.7–26 MPa), however, are much greater as compared to the chitosan film attached through the silanation reaction [16].

3-Aminopropyltriethoxysilane (APTES) is one silane molecule commonly used in the biomedical literature to bond an assortment of materials because of the primary amine group [8–10]. However in order to actually bond chitosan and titanium, a linker molecule, such as gluteraldehyde, must be used to modify the terminal amine group to an aldehyde group [16,29,30]. In previous research, a three-step process was used to bond chitosan to titanium, which qualitatively improved the bond strength [30]. One way to reduce the time required to attach a coating is to reduce the number of steps. Through careful selection of a silane molecule, the three-step process was reduced to a two-step process. As with previous research, toluene was used as the carrier solvent [30]. By using toluene as the solvent instead of an aqueous solution, loss of the reactive terminal groups and formation of the polysiloxane layer has been prevented [31,32].

The issue with linker molecules and the solvent used to deposit these molecules is not the only issue, however. Titanium is commonly used as an implant metal because it can become highly unreactive through a process called passivation [33]. This unreactive surface is highly desirable in the human body, as it prevents negative reactions [10]. However, this unreactive surface can also reduce the ability to bond a coating to the titanium implant. Piranha is a method to remove any carboneous materials [34], which may be introduced to the titanium surface because of the manufacturing and passivation processes [33]. Piranha has also been shown to etch titanium, which can help produce more surface area for the linker molecules to bond to the surface. Because of piranha's ability to react with both carbon and titanium, it is believed that the piranha-treated surface will have more reactive areas, thereby increasing the amount of linker molecules bound to the titanium surface and increasing coating bond strengths.

Following each reaction step, X-ray photoelectron spectroscopy (XPS) was used to determine if the chemical changes in the titanium surfaces were consistent with the anticipated reaction series. The effect of using toluene as the solvent on the reactive terminal aldehyde groups and the amount of TESBA bound by the two treated titanium surfaces were also examined using XPS. Therefore, the aim of this study was to evaluate the surface chemistry involved in the deposition of a covalently bound chitosan film on implant quality titanium using triethoxsilylbutyraldehyde (TESBA).

2. Experimental

2.1. Reagents

99.7+% ACS grade glacial acetic acid, gluteraldehyde, 35% aqueous solution hydrogen peroxide, 95–98% ACS grade sulfuric acid, 99% min. semiconductor grade toluene, and HPLC grade ultra-pure water were purchased from Alfa Aesar (Ward Hill, MA). 99.5% ACS grade acetone and 200 proof ethanol were purchased from Sigma–Aldrich (St. Louis, MO). ACS grade nitric acid and ACS grade isopropyl alcohol were purchased from Acros Chemical (Morris Plains, NJ). TESBA was purchased from Gelest (Morrisville, PA). Chitosan with a 86.4% degree of deacetylation (DDA) was obtained from Vanson (Redmond, WA). Deionized water was created using a NANOpure Diamond ultrapure water system (Barnstead, Boston, MA) with a D3750 hollow fiber filter with a maximum operating pressure of 50 psi and a 0.2 μm pore size rating.

2.2. Materials

A titanium bar with nominal dimensions of 3 in. × 5 in. × 0.25 in. was purchased from Titanium Industries (Jacksonville, FL) and cut into 1 in. × 1 in. × 0.25 in. coupons using a Makita Cut-Off saw with a carbide blade (La Mirada, CA).

2.3. Metal polishing

The steps involved in polishing the titanium metal coupons to a 1200 grit finish, were modified from a procedure previously used at Mississippi State University [35]. An electric belt sander (BR300, Type 1, Black and Decker, Towson, MD) with a grit of 120, width of 3 in. × 18 in., and speed of 656 ft/min was used to smooth out the roughest areas of the metal coupons. Next, 320 grit sandpaper (Norton, Worchester, MA) was used on a compressed air, dual action sander (Nikota, Whitter, CA) to remove the scratches made from the coarse grit and to continuing the smoothing process. The samples were then sanded by hand for the remainder of the polishing with 600, 800, and finally 1200 grit sandpaper. The coupons were sanded in one direction, rotated 90°, and again sanded in one direction. Sanding continued from coarser to finer grit until all residual scratches had been removed (determined by visual inspection).

2.4. Metal preparations

One of two methods of chemical cleaning, passivation or piranha, was performed on the polished metal coupons before a reaction series, but never both on the same sample.

2.4.1. Passivation method

Passivation was performed following the ASTM F86 standard [33]. The coupons were sonicated for 10 min in each of the following chemicals: acetone (70% by volume), then ethanol, and followed by deionized water. Following sonication in deionized water, the coupons were placed in a 3:7 (v/v) nitric acid–deionized water solution for 30 min at room temperature. Following the nitric acid treatment, the samples were rinsed with deionized water and placed in a covered ultra-pure water bath for 24 h.

2.4.2. Piranha treatment

The second chemical treatment method, piranha treatment, can be extremely dangerous. The coupons were first sonicated for 30 min in 70% isopropyl alcohol. Following sonication, concentrated sulfuric acid was poured into a beaker and 35% hydrogen peroxide slowly added at a 7:3 (v/v) ratio of sulfuric acid to hydrogen peroxide. The resulting mixture was then swirled gently to mix before being poured over the metal coupons. The coupons were left for 10 min before being removed and placed in a second piranha mixture for 5 min. Care should be taken remove the samples from the piranha solutions after 10 min and after 5 min, respectively. Piranha does react with the titanium and will etch the surface if the samples are left in the piranha solution for extended periods [34]. After the second piranha treatment, the metal coupons were rinsed twice in ultra-pure water before being placed in a covered ultra-pure water bath for 24 h.

2.5. Triethoxsilylbutyraldehyde and chitosan deposition

The following two-step silane deposition procedure was developed during this research to efficiently and effectively bind chitosan to titanium surfaces. Fig. 1 shows the anticipated reaction steps. Reaction step 1 is the deposition of TESBA on the titanium surface and reaction step 2 is the reaction between TESBA and chitosan.

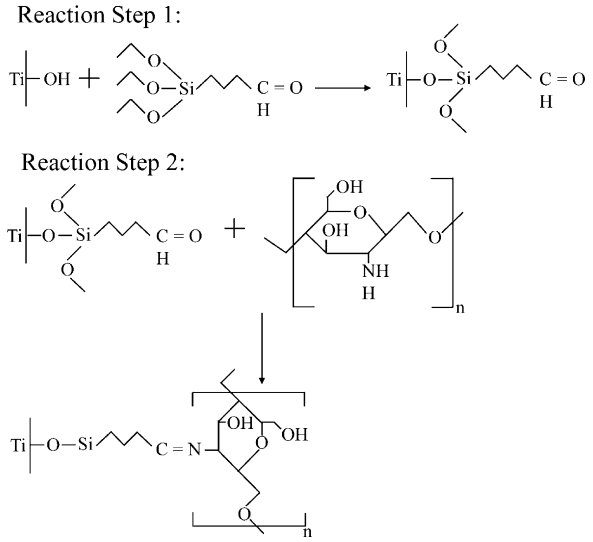

Fig. 1. Reaction steps involved in the binding of chitosan to titanium substrates: (1) triethoxsilylbutyraldehyde (TESBA) deposition; (2) reaction of TESBA with chitosan.

In the silane deposition step, dried titanium coupons (either passivated or piranha-treated) were submerged in a 2% (v/v) solution of TESBA in toluene in sealed individual containers and allowed to react for 24 h. Following the 24 h reaction time, the metal coupons were placed in pure toluene and sonicated for 30 min. The procedure of using fresh toluene with 30 min of sonication was repeated twice more, for a total sonication time of 90 min. To remove any residual toluene, the metal coupons were rinsed with ethanol followed by deionized water and then dried. Following the rinsing and drying process, the coupons were stored in individual containers.

The second step in the reaction series involved the chitosan film deposition and was developed by Bumgardner et al. [16]. A solution of 1 wt.% chitosan, 2 wt.% acetic acid, and 97 wt.% deionized water was prepared. The solution was stirred for 1 h to ensure that the chitosan had dissolved and then filtered through several layers of cheesecloth to remove any undissolved particulate. The filtered chitosan solution was poured over the metal coupons in the Petri dishes. The solution was then allowed to evaporate for 7–10 days; after which time, a clear film was seen on the surface of the metal coupons (as the reflection of light was different than on an untreated metal coupon).

2.6. X-ray photoelectron spectroscopy

A PHI 1600 XPS Surface Analysis System (Physical Electronics, Eden Prairie, MN) was used to obtain XPS data from an area approximately 800 μm in diameter. The instrument also uses a PHI 10-360 spherical capacitor energy analyzer and an Omni Focus II small-area lens to focus the incident electron beam. XPS data were obtained using an

achromatic Mg Kα X-ray source operating at 300 W and 15 kV. Survey spectra were gathered using an average of 10 scans with a pass energy of 26.95 eV and running from 1100 to 0 eV. High-resolution spectra were gathered using an average of 15 scans with a pass energy of 23.5 eV and a step size of 0.1 eV. The incident sample angle was held constant at 45°. Gold foil was used to calibrate the binding energy, using a peak assignment of $4f_{7/2}$ at 84.0 eV. To determine charge effects, the carbon 1s peak was used for reference, with adventitious carbon assigned to 284.5 eV. No charge effects were seen for these samples. For statistical analysis, measurements were taken on three samples per treatment and three spots per sample, producing nine statistical data points.

The XPS data was collected and averaged using PHI Surface Analysis Software (Version 3.0, Physical Electronics, Eden Prairie, MN). The XPS data was then analyzed using the Spectral Data Processor (SDP) (Version 4.0, XPS International LLC, Mountain View, CA). Statistical analyses were performed using SAS software (Version 9.1, SAS Institute Inc., Cary, NC). Comparison of the individual reaction steps was performed using a completely randomized design with subsampling.

3. Results and discussion

The samples were scanned using XPS after each reaction step. The passivated and piranha-treated titanium surfaces were first scanned using XPS. TESBA was then deposited (Fig. 1, reaction step 1) and XPS was performed on the TESBA treated surfaces. The chitosan film was then deposited (Fig. 1, reaction step 2) and XPS was run on the final film.

3.1. XPS analysis of the triethoxsilylbutyraldehyde deposition

Table 1 shows the differences in the normalized elemental peak areas following the two metal treatments and following the TESBA deposition (reaction step 1). Prior to the TESBA deposition, the composition of oxygen and titanium were significantly higher on the piranha-treated surface as compared to the passivated surface, while the composition of carbon was significantly lower on the piranha-treated surface. No silicon was present on either of the two titanium surfaces prior to reaction step 1. Following the TESBA deposition, the amounts of carbon, oxygen, and silicon were statistically equivalent. There was more titanium present on the passivated surface as compared to the piranha-treated surface.

Table 1
Elemental peak areas on the treated titanium surfaces from XPS survey scans (per unit area)

Element	Passivated	TESBA	Piranha-treated	TESBA
Carbon	1.00 ± 0.06 a	1.00 ± 0.07 ab	0.64 ± 0.03	1.04 ± 0.05 b
Oxygen	1.13 ± 0.08	1.58 ± 0.16 c	1.77 ± 0.07	1.46 ± 0.14 c
Silicon	–	0.30 ± 0.03 d	–	0.29 ± 0.01 d
Titanium	0.60 ± 0.24	0.11 ± 0.07	1.71 ± 0.29	0.03 ± 0.05

Values with the same letter are not statistically different at the 5% significance level. XPS was performed on three samples per treatment, with three spots per sample, producing nine data points. All values are normalized based on the passivated carbon peak, where the XPS intensity of the element of interest is divided by the XPS intensity of the passivated carbon peak.

The higher amounts of oxygen and titanium on the piranha-treated surface as compared to the passivated surface are likely the result of the treatment method. Piranha treatment has been shown to etch titanium [34], which would likely create a larger surface area. This probable increase in surface area would increase the amount of oxygen that could be bound when the titanium coupons were placed in the ultra-pure water bath. Also, passivation is designed to create a layer of unreactive titanium that is several atomic layers thick [10], which could ultimately prevent attachment of TESBA molecules to the titanium surface. The piranha treatment and the ultra-pure water bath should produce fewer unreactive groups than the passivated treatment. While titanium does react with air to create an unreactive layer, this layer would not likely be as thick as the unreactive layer created by passivation. This difference in thickness would allow for more reactive sites to bond the TESBA molecules.

The higher amount of carbon on the passivated titanium surface as compared to the piranha-treated surface is also likely the result of the treatment method. The passivation method uses solvents in the cleaning phase, which would result in the deposition of carbon. These carbon deposits were not removed by the nitric acid during the passivation phase. Also, piranha is regularly used to remove carbon, as it reacts strongly with carboneous materials [34], which would remove adventitious carbon from the piranha-treated titanium surfaces.

Following the TESBA deposition, the amount of carbon, oxygen, and silicon were statistically similar, while titanium was higher on the passivated surface compared to the piranha-treated surface. Silicon was present following sonication, demonstrating that the silane molecules were chemically bound to the titanium surface and not physiosorbed. By examining the amount of titanium, silicon, and oxygen, one can determine that more TESBA is bound to the piranha-treated titanium as compared to the passivated titanium.

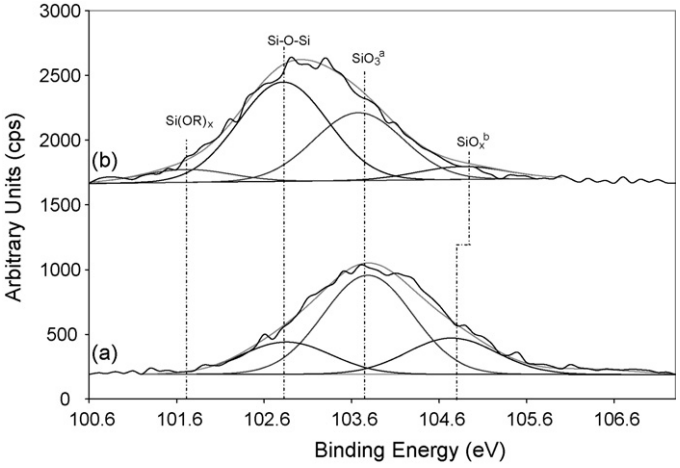

Fig. 2. XPS high-resolution spectra of silicon following TESBA deposition: (a) passivated titanium surface; (b) piranha-treated titanium surface. Peaks with the same superscript are not statistically different at the 5% significance level.

There were four silicon species present following the TESBA deposition, as shown in Fig. 2. One peak, $Si(OR)_x$ [36], was present only on the piranha-treated surface, while one other peak, SiO_3 [37], was statistically equivalent for both the passivated and piranha-treated surfaces. Both $Si(OR)_x$ and SiO_3 are present because of the triethoxy ends of the silane molecule. Examining Fig. 2, there appears to be a shift in the SiO_x peak between the passivated and piranha-treated surface. This shift is not actually significant, but instead was within the range of 105.4 ± 0.3 eV. The Si–O–Si [38] peak was significantly higher on the piranha-treated surface as compared to the passivated surface (1010 ± 410 cps vs. 700 ± 370 cps). The presence of the Si–O–Si peak demonstrates that the formation of interlinking silane molecules over the metal surface developed when two or more adjacent silanes react with one another via residual ethoxy groups [39]. The ability of the adjacent silane molecules to form Si–O–Si over the piranha-treated titanium surface suggests that the TESBA groups were packed more closely together, an indication that more TESBA groups were bound to the titanium surface.

The closely packed TESBA groups on the piranha-treated surface is further demonstrated by the titanium high resolution scan, as shown in Fig. 3. There are only two species of titanium following the deposition of TESBA, TiO [40] and TiO_2 [41]. However, there is significantly more titanium, in the form of TiO, present on the passivated surface (500 ± 300 cps) as compared to the piranha-treated surface (170 ± 150 cps), as shown in both Table 1 and Fig. 3. There appears to be shift in the peak location of TiO_2. As with SiO_3, this shift is not significant, but instead is within the range of 459.5 ± 0.4 eV. The smaller amount of titanium present on the piranha-treated surface further demonstrates that the TESBA groups were more closely packed. The bound layer of TESBA is preventing the exit of the titanium photoelectrons, thereby reducing the titanium signal. On the passivated titanium surface, the TESBA layer is not as closely packed, thereby allowing more titanium photoelectrons to be detected.

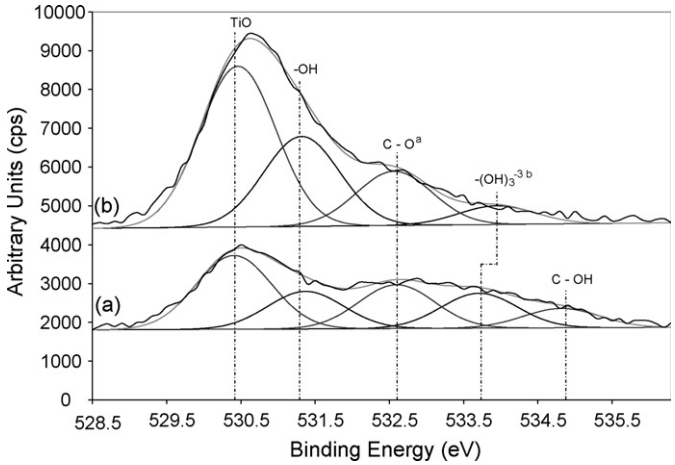

Fig. 4. XPS high-resolution spectra of oxygen following surface treatment: (a) passivated titanium surface; (b) piranha-treated titanium surface. Peaks with the same superscript are not statistically different at the 5% significance level.

The difference in the TESBA layer deposition is further confirmed when the titanium surface before and after deposition is examined. Fig. 4 shows the oxygen peak from the two titanium surfaces following the surface treatment but prior to the TESBA deposition, while Fig. 5 shows the oxygen peak from the two titanium surfaces following TESBA deposition. As shown in Fig. 4, the piranha-treated titanium surface has significantly higher concentrations of TiO [42] and –OH [43] than the passivated titanium surface. In fact, the concentrations of TiO on the piranha-treated surface, 6970 ± 250 cps, as compared to the concentration on the passivated surface, 3330 ± 790 cps, is more than double. The concentration of –OH on the piranha-treated surface compared to the passivated surface shows the same trend, with more than double the amount of the –OH group on the piranha-treated surface as compared to the passivated surface, with 3830 ± 350 cps compared to 1920 ± 200 cps, respectively. Following the deposition of TESBA, the amount of TiO on both titanium surfaces is identical, indicating that more of the

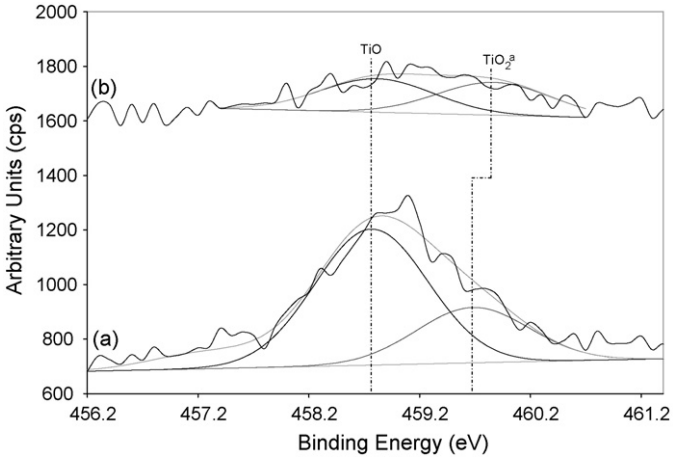

Fig. 3. XPS high-resolution spectra of titanium following TESBA deposition: (a) passivated titanium surface; (b) piranha-treated titanium surface. Peaks with the same superscript are not statistically different at the 5% significance level.

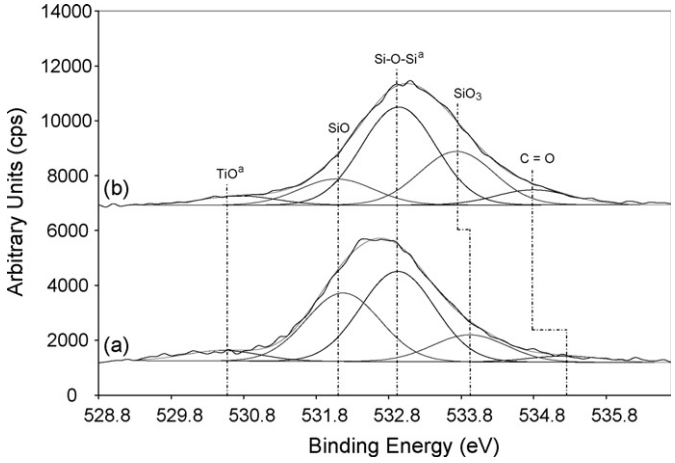

Fig. 5. XPS high-resolution spectra of oxygen following TESBA deposition: (a) passivated titanium surface; (b) piranha-treated titanium surface. Peaks with the same superscript are not statistically different at the 5% significance level.

titanium is covered on the piranha-treated surface. The silicon species, Si–O–Si [38], is also statistically identical on both surfaces. The presence of Si–O–Si across both surfaces indicate that the silane molecules are linking to neighboring molecules, producing a polysiloxane layer. However, Si–O–Si is not the only indicator of a polysiloxane layer, which can be used to determine which surface has more silane molecules. The silicon species, SiO_3 [44], exist in significantly higher concentrations on the piranha-treated surface as compared to the passivated surface. In fact, the concentration of SiO_3 on the piranha-treated surface is almost double the concentration of SiO_3 on the passivated surface, 3420 ± 730 cps compared with 1680 ± 450 cps. The presence of SiO_3 on the surface of the piranha-treated surface indicate that there are more O–Si–O bonds, demonstrating that there are more silane molecules on the piranha-treated surface, which would allow the silane molecules to link with neighboring silane molecules. In contrast to SiO_3, SiO [45] occurs at more than double the concentration on the passivated surface as compared to the piranha-treated surface, 4000 ± 1160 cps compared with 1650 ± 830 cps. While Si–O–Si is present in equal amounts on both surfaces, the higher amount of SiO on the passivated surface indicated that there was more space between some of the TESBA molecules. The examination of the silicon–oxygen species demonstrated that there was likely more TESBA bound to the piranha-treated surface as compared to the passivated surface. The quantification of the aldehyde group, C=O, which is necessary for the chitosan to bond to the titanium surface, further supports the theory that more TESBA molecules are bound to the piranha-treated surface. When comparing the passivated surface to the piranha-treated surface, there are significantly more C=O [46] groups on the piranha-treated surface, 1310 ± 360 cps for the piranha surface as compared to 370 ± 90 cps for the passivated surface. While there are some C=O groups on the passivated surface, the amount is very small, indicating that there are few places for the chitosan to bond to the passivated surface. There does appear to be shift in the peak location of C=O. As with SiO_3 and TiO_2, this shift is not significant, but instead is within the range of 534.9 ± 0.3 eV.

3.2. XPS analysis of the chitosan films

The chitosan films remained attached to the titanium surface while under the ultra-high vacuum required for XPS [47]. Qualitatively, this demonstrated that the bond between the chitosan film and the metal surface was greatly improved over the previously published method [15], since coatings made by the previous method were unable to remain attached to the titanium surface in the ultra-high vacuum environment. No effect to the qualitative bond strength was seen by using the two-step process (deposition of TESBA) as compared to the three-step process (deposition of APTES). The lack of significant differences between the passivated metal surface and the piranha-treated metal surface was previously covered [30]. Briefly, there were no significant differences between the two titanium surfaces with respect to carbon, oxygen, nitrogen, silicon, or calcium [30]. The lack of differences between the two surfaces demonstrated that there were no significant changes in the chemical composition of the chitosan films due to the metal treatments. Table 2 shows the differences between the normalized elemental peak areas of the chitosan films attached to the treated metal surfaces based on the two silane molecules used (APTES and TESBA). There were no significant differences between the two films based on the composition of nitrogen and calcium. However, there were significant differences between carbon, oxygen, silicon, and phosphorous. The significant differences in silicon and phosphorous may be due to the small sampling size and/or possible variations in the silicon and phosphorous concentrations for the chitosan aliquots pulled for preparation of each chitosan solution. The differences in silicon and phosphorous concentrations can also be attributed to the fact that chitin, used to created chitosan, is formed biologically, resulting in slight variability among different shellfish. The differences in the concentrations of both oxygen and carbon again could be contributed to the small sampling size. Since the films are approximately 100 μm thick, as determined by visual examination, and XPS can penetrate at most 10 nm into the sample, no conclusions about the bonding of the chitosan film to TESBA can be made from the XPS data [48]. The small sampling depth could also result in the differences seen in the oxygen and carbon concentrations. The addition of a bulky linker molecule, gluteraldehyde, with APTES will change the density of the chitosan chains. Differences in polymer chain density between the APTES and TESBA modified titanium could lead to differences in packing and surface conformation. This could cause different chemical groups to be positioned near the surface and result in different concentrations of oxygen and carbon. While there were differences in the concentrations of oxygen and carbon, the differences were small, indicating that there were no changes to the chitosan film based on the silane treatment used.

Table 2
Elemental peak areas of the chitosan films from XPS survey scans (per unit area)

Element	APTES	TESBA
Carbon	1.00 ± 0.03	1.06 ± 0.04
Oxygen	0.97 ± 0.09	1.05 ± 0.07
Nitrogen	0.14 ± 0.02 a	0.14 ± 0.03 a
Calcium	0.06 ± 0.03 b	0.09 ± 0.03 b
Phosphorous	0.01 ± 0.01	0.02 ± 0.01
Silicon	0.01 ± 0.01	0.06 ± 0.03

Values with the same letter are not statistically different at the 5% significance level. XPS was performed on three samples per treatment, with three spots per sample, producing nine data points. All values are normalized based on the APTES carbon peak, where the XPS intensity of the element of interest is divided by the XPS intensity of the APTES carbon peak.

4. Conclusions

XPS was used to document the deposition of TESBA on two different titanium surfaces. There were significant changes in the amounts of oxygen, carbon, silicon, and titanium detected

by XPS, which were consistent with the anticipated reaction steps. It was demonstrated that more TESBA was bound to the piranha-treated surface as compared to the passivated surface, based on the lower amount of titanium and the higher amount certain species of silicon. XPS was also used to document the attachment of chitosan to the titanium surface via the linker TESBA molecules. The chitosan films produced using the two-step process presented in this research remained attached when stressed in the ultra-high vacuum required for XPS, qualitatively indicating that the chitosan films were tightly bound. The chitosan films produced using the TESBA deposition were compared with the chitosan films produced using the APTES deposition. The differences in the oxygen and carbon compositions of the chitosan films are likely the result of differing surface conformations of the chitosan chains and not changes in the chitosan film chemistry.

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