Grafting titanium nitride surfaces with sodium styrene sulfonate thin films

Gilad Zorn^{a)}

National ESCA and Surface Analysis Center for Biomedical Problems, Departments of Chemical Engineering and Bioengineering, University of Washington, Seattle, Washington 98195-1653

Véronique Migonney

Laboratory of Biomaterials and Specialty Polymers (LBPS/CSPBAT - UMR CNRS 7244), Institut Galilée, Université Paris 13 Sorbonne Paris Cité, 93430 Villetaneuse, France

David G. Castner

National ESCA and Surface Analysis Center for Biomedical Problems, Departments of Chemical Engineering and Bioengineering, University of Washington, Seattle, Washington 98195-1653

(Received 4 April 2014; accepted 6 May 2014; published 21 May 2014)

The importance of titanium nitride lies in its high hardness and its remarkable resistance to wear and corrosion, which has led to its use as a coating for the heads of hip prostheses, dental implants and dental surgery tools. However, the usefulness of titanium nitride coatings for biomedical applications could be significantly enhanced by modifying their surface with a bioactive polymer film. The main focus of the present work was to graft a bioactive poly(sodium styrene sulfonate) (pNaSS) thin film from titanium nitride surfaces via a two-step procedure: first modifying the surface with 3-methacryloxypropyltrimethoxysilane (MPS) and then grafting the pNaSS film from the MPS modified titanium through free radical polymerization. X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) were used after each step to characterize success and completeness of each reaction. The surface region of the titanium nitride prior to MPS functionalization and NaSS grafting contained a mixture of titanium nitride, oxynitride, oxide species as well as adventitious surface contaminants. After MPS functionalization, Si was detected by XPS, and characteristic MPS fragments were detected by ToF-SIMS. After NaSS grafting, Na and S were detected by XPS and characteristic NaSS fragments were detected by ToF-SIMS. The XPS determined thicknesses of the MPS and NaSS overlayers were ~ 1.5 and ~ 1.7 nm, respectively. The pNaSS film density was estimated by the toluidine blue colorimetric assay to be 260 ± 70 ng/cm². © 2014 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4878215>]

I. INTRODUCTION

Titanium nitride (TiN) has excellent corrosion and wear resistance properties and has been used as a hard coating on titanium surgical implants such as hip prostheses and dental implants.^{1–5} Although titanium nitride is sufficiently bioinert to allow direct apposition to tissue when placed in the body, a robust implant–tissue interface will not be developed unless the material has the ability to guide initial cellular reactions at the implant site. In general, titanium implants can induce the formation of a fibrous layer that compromises bonding at the interface with the living tissue. This lack of proper integration of titanium with tissue can lead to implant failure by aseptic loosening.⁶ Since the biological response to implanted biomaterials is influenced by the surface properties of the materials, modifying the chemical properties of the implant's surface can often improve the control of the interactions between an implant and its surrounding tissue.^{7,8}

A promising simple approach to modify the surface of titanium implants is grafting a bioactive polymer from their surfaces.⁷ Atomic transfer radical polymerization (ATRP) grafting is a common method often used since it does not require stringent experimental conditions.^{9–11} It is an

effective method of grafting polymers from titanium surfaces.^{12–17} More recently, radical polymerization grafting was improved by the reversible addition-fragmentation chain-transfer (RAFT) grafting method to allow the direct polymerization of a wide range of functional monomers in a controlled fashion with narrowly dispersed molecular weight.^{18–22} It has already been shown that polymers bearing appropriate chemical functions can modulate cell attachment, spreading, and activity on these bioactive polymers.^{23–26} The distributions of ionic groups along with the macromolecular chains create active sites, which can interact with extracellular proteins, such as fibronectin, implicated in cell response.^{23–27} Given the benefits of titanium nitride in terms of wear resistance and corrosion protection, grafting of a bioactive polymer from the titanium nitride surface is a potentially useful way to combine the benefits of titanium nitride with the grafted bioactive polymer.

A successful grafting from the titanium nitride surface requires the use of a bifunctional molecule that has one group that can be covalently attached to the surface and another group that can be used for radical polymerization. For titanium and titanium alloys, successful attachment has been reported using both siloxane and phosphonate “head groups.”^{13,16,27–30} A recent study showed that phosphonic acids would not form a robust film directly onto the native surface of titanium nitride and additional surface oxidation

^{a)}Present address: General Electric Global Research One Research Circle, Niskayuna, New York 12309. Author to whom correspondence should be addressed; electronic mail: zorn@ge.com

of the titanium nitride was required to form robust films.³¹ Surface modification of titanium nitride is affected by its outermost surface, which is a mixture of titanium dioxide and titanium oxynitride.^{1,31–34} It was suggested that covalent bonding and self-assembly of phosphonate molecules onto the surface of titanium oxide is possible due to surface OH group regeneration via proton transfer from the phosphonic group to the surface oxygen.³⁵ However, in the case of titanium nitride surface, OH group regeneration only occurs when there is an oxygen atom close to the phosphonic hydrogen, whereas no such regeneration is possible if the next neighbor is nitrogen. Siloxane chemistry has been widely used to anchor polymers such as styrene, poly(ethylene glycol)acrylate, and poly(ethylene glycol)methacrylate onto titanium oxide surfaces through ATRP.^{16,17,27} A recent study showed that siloxane chemistry could be used to graft of bioactive poly(sodium styrene sulfonate) (pNaSS) from titanium oxide surfaces.²⁷ Silanes require hydroxylated surfaces to form coatings. The driving force for this self-assembly is the *in situ* formation of polysiloxane with the Si-O bond connected either to another polysiloxane chain or to the surface.^{35,37,38} The difference in covalent bonding and self-assembly between phosphonic acids and silanes makes the latter more suitable for tailoring the surface of titanium nitride. However, given the different reactivities of the titanium oxide and nitride surfaces, it is essential to carefully characterize the functionalization process on both surfaces. To our knowledge, silanes have not been used to graft bioactive polymers from titanium nitride surfaces and hence form the basis for this study.

Thus, the main thrust of the present work was to evaluate whether the two-step approach of siloxane modification and pNaSS grafting, which was developed for titanium oxide surfaces,²⁷ can also be applied to biomodify the surface of titanium nitride. The success of this two-step approach was evaluated on a smooth model system of evaporated titanium oxide. This smooth model allowed utilization of a wide range of analytical techniques for detailed characterization of the grafted films.²⁷ Unlike model systems, studying and characterizing thin films grafted onto the surface of real world biomaterials is challenging, mainly due to their surface roughness.

In this work, the surfaces of real world titanium nitride were first modified by attaching a monolayer of 3-methacryloxypropyltrimethoxysilane (MPS). Then the pNaSS films were grafted from the modified surface using free radical polymerization to bond the methacrylate tails of the siloxane monolayer to the carbon-carbon double bond ($C=C$) of the NaSS monomers.²⁷ Surface roughness was measured with atomic force microscopy (AFM). Quantitative surface elemental compositions after each step were determined with x-ray photoelectron spectroscopy (XPS). XPS was also used to estimate the thickness of the organic overlayers on the surface of the titanium nitride. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) provided molecular structure information about the MPS and NaSS layers. Toluidine blue colorimetric assay provided the surface density of the grafted

NaSS groups. These methods were found to be suitable for characterizing organic overlayers on a real world biomaterial with high surface roughness and together, they provide complementary surface characterization of the pNaSS films grafted from the titanium nitride surfaces. Unlike the differences observed for phosphonic acid functionalization of titanium oxide and nitride films, the silane functionalization was observed to be similar on the two different surfaces.

II. MATERIALS AND METHODS

A. Materials

Titanium foil coated with a 2–3 μm titanium nitride layer was purchased from Goodfellow (Oakdale, PA). MPS was purchased from Gelest, Inc. (Morrisville, PA). Sodium styrene sulfonate (NaSS), azobisisobutyronitrile (AIBN), chloroform (CHCl_3), dimethyl sulfoxide (DMSO), toluidine blue, 2-amino-2-methyl propanol, NaOH, and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). NaSS was purified by dissolving it in a mixture of water/ethanol (10/90 v/v) at 70 °C and then recrystallizing it under vacuum.²⁵ AIBN was purified by dissolving it in methanol, recrystallizing it at –18 °C, and then drying under vacuum. All other chemicals and materials were used as received.

B. Silanization and NaSS grafting

Samples of $0.5 \times 0.5 \text{ cm}^2$ were cut from the titanium nitride coated titanium. The samples were sonicated in CHCl_3 for 15 min and soaked in a CHCl_3 solution of MPS (5% v/v) for 1 h.^{36,39} The samples were then gently rinsed in CHCl_3 followed by heating at 140 °C for 4 h.³⁶ Finally, the samples were sonicated in CHCl_3 for 30 min and then were air dried. After drying, the samples were immersed in a DMSO solution of 0.7 M NaSS monomer^{7,24,25} and 9×10^{-3} M AIBN. The solution was degassed for 2 h to create an oxygen-free atmosphere and then heated to 90 °C for 15 h under vacuum.²⁷ The samples were then rinsed in deionized (DI) water, sonicated for 15 min in DI water, and dried under vacuum.

C. Atomic Force Microscopy

The surface morphologies and roughness were measured using AFM (Dimension 3100, Veeco Metrology, Inc., Santa Barbara, CA, equipped with a Nanoscope IV controller). The AFM analyses were operated in the tapping mode in air with a 300 kHz, 40 N/m Vistaprobes silicon tip coated with aluminum (Nanoscience Instruments Phoenix, AZ). At least three locations on the sample were scanned to determine the average roughness over a 400 μm^2 area.

D. Toluidine blue colorimetric assay

The colorimetric assay procedure was adopted from previous studies that were performed by Migonney and co-workers.^{24,25} Briefly, 7.5 g of toluidine blue was dissolved in 50 ml DI- H_2O and a buffer solution of 0.2 ml of 2-amino-2-methyl propanol was added. Each polymerized sample was

placed in an individual well of a six-well plate with 6 ml of the solution and incubated at 30 °C for 6 h. The samples were then soaked in a 5×10^{-4} M NaOH solution for 30 min. Each sample was then placed in a new well with 5 ml of acetic acid (50% aq.) for 24 h. The remaining decomplexed solution was analyzed using a 96-well microplate reader (Tecan), and the polymer surface density was calculated as follows:^{24,25}

$$G(\text{grafted polymer [g]}) = CVM/2, \quad (1)$$

where C = molar concentration of the monomer in the decomplexed solution, V = acetic acid volume (0.005 l), and M = molar weight of the monomer (206 g/mol).

E. X-ray photoelectron spectroscopy

XPS measurements were performed on a Kratos AXIS Ultra DLD instrument (Kratos, Manchester, England) employing a hemispherical analyzer for spectroscopy. Spectra were acquired with a monochromated Al K_{α} X-ray source and a nominal 0° take-off angle (TOA). The TOA is defined as the angle between the sample surface normal and the axis of the XPS analyzer lens. Spectra were acquired with the analyzer hybrid mode (large analyzer acceptance angle mode with 700 $\mu\text{m} \times 300 \mu\text{m}$ field of view). A coaxial filament was used to produce low energy electrons for charge neutralization during all measurements. Compositional survey scans were acquired using pass energy of 80 eV, and high-resolution spectra were acquired using pass energy of 20 eV. All binding energies (BEs) were referenced to the hydrocarbon C1s peak at 285 eV. Three spots on two or more replicates of each sample type were analyzed. The compositional data are an average of the values determined at each spot. Compositions were calculated with the CasaXPS software and the high-resolution peaks were fit with the XPSPEAK 4.1 software.

F. Time of flight secondary ions spectroscopy

ToF-SIMS experiments were performed using an ION-TOF TOF.SIMS 5–100 (ION-TOF GmbH, Münster, Germany). A liquid metal ion gun (LMIG) was used to generate the pulsed 25 keV Bi_3^+ beam. The analysis Bi_3^+ beam was rastered over a $100 \times 100 \mu\text{m}^2$ area on the sample surface, and the total ion dose used to acquire each spectrum was less than 1×10^{12} ions/ cm^2 . A low energy electron flood gun was used for charge compensation during all measurements to stabilize surface charge and the high current bunched mode (i.e., high mass resolution or spectroscopy mode) was used for analysis. The mass resolution ($m/\Delta m$) at $m/z = 27$ was above 4000 for all mass spectra. The mass scales of the positive secondary ion spectra were mass calibrated using the CH_3^+ , C_2H_3^+ , C_2H_5^+ , and C_3H_5^+ peaks. The mass scales of the negative secondary ion spectra were mass calibrated using the CH^- , O^- , OH^- , and C_2H^- peaks. At least two replicates were analyzed for each sample type with five spectra acquired at five different spots on each replicate. Data analysis was performed using the IonSpec data

FIG. 1. AFM micrograph of $20 \mu\text{m} \times 20 \mu\text{m}$ of the as received titanium nitride surface.

reduction software from ION-TOF GmbH. Peak intensities were normalized using the total intensity for each spectrum.

III. RESULTS AND DISCUSSION

Prior to the pNaSS grafting process, the composition and structure of the titanium nitride surface was characterized. From the AFM image in Fig. 1, it was determined that the titanium nitride surface had an average roughness of ~ 230 nm over a $400 \mu\text{m}^2$ area. The XPS survey spectrum for the titanium nitride is shown in Fig. 2(a) and its corresponding surface composition is shown in Table I. As expected, the surface consisted of titanium, nitrogen, oxygen, and carbon. The ~ 35 at. % carbon likely arises from the presence of adventitious organic contamination on the surface.^{27,29,31} The

FIG. 2. XPS survey spectra of (a) bare titanium nitride, (b) MPS modified titanium nitride, and (c) pNaSS grafted titanium nitride.

TABLE I. XPS determined surface elemental compositions of bare titanium nitride, MPS modified titanium nitride, and pNaSS grafted titanium nitride.

	Bare titanium nitride (at. %)	MPS modified titanium nitride (at. %)	NaSS grafted titanium nitride (at. %)
Ti	15.2 ± 0.8	8.8 ± 0.5	4.5 ± 0.1
O	32.9 ± 0.4	34.3 ± 0.8	28.8 ± 0.2
C	34.8 ± 0.6	41.9 ± 0.2	50.5 ± 1.6
N	17.0 ± 0.3	10.2 ± 0.5	7.1 ± 0.4
Si	...	4.9 ± 0.4	3.3 ± 0.5
Na	...	...	2.6 ± 0.3
S	...	...	3.2 ± 0.2

high resolution C1s line shape of this adventitious organic contamination is shown in Fig. 3(d). The high resolution Ti2p3/2 line shape [Table II and Fig. 3(a)] was fit to three components at 455.3, 456.8, and 458.3 eV, corresponding to presence of titanium nitride, titanium oxynitride and titanium dioxide, respectively, in the surface region.^{1,32–34} The high resolution O1s line shape [Table II and Fig. 2(g)] was fit to three components at 530.2 eV (TiO₂), 531.8 eV (titanium oxynitride and adventitious organic contamination), and 533.1 eV (surface hydroxide groups and adsorbed water, as well as adventitious organic contamination).

The ToF-SIMS spectra collected from the bare titanium nitride are shown in Figs. 4(a) and 5(a). Considering the ToF

SIMS high surface sensitivity with a sampling depth of 2–3 nm, the main fragments that were detected in these spectra are attributed to the adventitious surface contamination. Still, Ti_xO_yN_z and Ti_xO_y fragments could also be detected from the bare titanium nitride surface in positive and negative ToF-SIMS (Tables III and IV). These species observed by ToF-SIMS are consistent with the XPS results and provide further support for the presence titanium oxynitride on the titanium nitride surface. In addition, the Ti_xO_yH_z fragments that were detected in ToF-SIMS negative secondary ion spectra (Table IV) are consistent with the presence of hydroxide groups on the titanium nitride surfaces, which are essential for surface silanization.^{27,35,37}

Siloxane-anchored thin films are known to interact strongly on a variety of surfaces^{38,40} such as silicon oxide,^{41–43} aluminum oxide,^{44,45} glass,⁴³ and titanium oxides.^{16,17,27} Therefore, siloxane chemistry was chosen to modify the titanium nitride surfaces. After attaching the MPS molecules onto the titanium nitride surfaces, the XPS surface composition detected the presence of Si, which is an element unique to the MPS trimethoxysilane tails [Table I and Fig. 2(b)]. The carbon to silicon ratio in Table I is 8.6, which is slightly higher than the theoretical value of 7 expected for a fully hydrolyzed and crosslinked MPS molecule (i.e., elimination of all methyl groups from all three methoxy species in the starting MPS molecule). The slightly larger experimental value indicates either all the methoxy groups are not

Fig. 3. High-resolution XPS Ti2p spectrum of (a) titanium nitride, (b) MPS modified titanium nitride, and (c) NaSS grafted titanium nitride. High-resolution XPS C1s spectra of (d) titanium nitride, (e) MPS modified titanium nitride, and (f) NaSS grafted titanium nitride. High-resolution XPS O1s spectra of (g) titanium nitride, (h) MPS modified titanium nitride, and (i) NaSS grafted titanium nitride.

TABLE II. High resolution XPS peak fitting results for the Ti2p3/2, O1s and C1s spectra shown in Fig. 3 from bare titanium nitride, MPS modified titanium nitride, and pNaSS grafted titanium nitride.

	BE	Possible species	Fractional peak area		
			Bare titanium nitride	MPS modified titanium nitride	NaSS grafted titanium nitride
Ti2p	455.3	TiN	0.34	0.25	0.24
	456.8	TiO _x N _y	0.24	0.29	0.28
	458.3	TiO ₂	0.42	0.46	0.48
O1s	530.2	TiO ₂	0.51	0.44	0.21
	531.8	TiO _x N _y Ti-O-H C=O S=O	0.27	0.34	0.51
	533.1	C-O-C=O	0.22	0.22	0.27
C1s	285.0	C-C; C-H	0.70	0.60	0.84
	286.5	C-O-C C-O-Si	0.20	0.24	0.16
	288.9	O-C=O	0.10	0.15	<0.1

FIG. 4. Positive secondary ion fragments detected by ToF SIMS analysis of (a) bare titanium nitride and (b) MPS modified titanium nitride.

FIG. 5. Negative secondary ion fragments detected by ToF SIMS analysis of (a) bare titanium nitride, (b) MPS modified titanium nitride, and (c) pNaSS grafted titanium nitride.

TABLE III. Relative intensities of positive secondary ion fragments detected in ToF-SIMS analysis of the bare titanium nitride, MPS modified titanium nitride, and pNaSS grafted titanium nitride.

Ion	Mass		Bare titanium nitride	MPS modified titanium nitride	NaSS grafted titanium nitride
Ti ⁺	47.946	TiN	2.71	2.59	0.47
TiN ⁺	61.954		0.53	0.51	0.15
TiO ⁺	63.941		2.12	1.86	0.35
TiON ⁺	77.952		0.03	0.04	0.01
TiNO ₂ ⁺	93.946		0.06	0.04	0.02
Ti ₂ O ₂ ⁺	127.879		0.13	0.08	0.02
Ti ₂ N ₃ ⁺	137.900		0.07	0.04	0.01
Ti ₂ O ₃ ⁺	143.870		0.29	0.20	0.05
Si ⁺	27.975	MPS	Background level	0.45	0.32
C ₂ H ₃ O ⁺	43.017			3.00	1.78
C ₄ H ₅ O ⁺	69.036			2.20	0.40
TiO ₂ Si ⁺	107.909			0.14	0.05

hydrolyzed or that some adventitious hydrocarbon species or organic solvent residues are present on the MPS modified titanium nitride. The attenuation of the titanium and nitrogen signals by

the MPS overlayer can be used to calculate the overlayer thickness using⁴⁶

$$I = I_0 e^{-d/\lambda \cos \theta}. \quad (2)$$

In Eq. (2), λ is the inelastic mean free path of the Ti2p or N1s photoelectrons through the organic overlayers (~ 2.2 nm for Ti2p and ~ 2.4 nm for N1s);⁴⁷ θ is the photoelectron take-off angle from the surface normal; I is the Ti2p or N1s intensity from the MPS modified titanium nitride; and I_0 is intensity from bare clean unmodified titanium nitride. Since the surface of the bare titanium nitride has ~ 35 at. % adventitious carbon contamination, the measured Ti2p or N1s intensities from the bare titanium nitride must be corrected first for the attenuation from the adventitious carbon overlayer. To determine the Ti2p and N1s I_0 values for a clean titanium nitride surface first the thickness of the contamination layer, d_{Carbon} , was estimated using the following equation⁴⁸ with λ_{Carbon} ≈ 3.0 nm (Refs. 49 and 50) to be 1.3 nm:

$$d_{\text{Carbon}} = -\lambda_{\text{Carbon}} \cos \vartheta \ln \left(\frac{100 - \%C_{\text{Contamination}}}{100} \right). \quad (3)$$

TABLE IV. Relative intensities of negative secondary ion fragments detected in ToF-SIMS analysis of the bare titanium nitride, MPS modified titanium nitride, and pNaSS grafted titanium nitride.

Ion	Mass		Bare titanium nitride	MPS modified titanium nitride	NaSS grafted titanium nitride
TiO ₃ H ⁻	96.952	TiN	1.48	0.81	0.47
TiO ₃ H ₂ ⁻	97.956		0.30	0.13	0.07
TiO ₂ N ₂ ⁻	107.950		0.09	0.04	0.04
TiO ₂ N ₃ ⁻	121.941		0.17	0.12	0.08
C ₃ H ₃ O ⁻	55.020	MPS	Background level	0.34	0.15
C ₃ H ₅ O ⁻	57.038			0.15	0.09
SiO ₂ ⁻	59.970			1.53	0.85
SiHO ₂ ⁻	60.978			1.60	1.16
SiCH ₂ O ₂ ⁻	73.991			0.11	0.07
C ₄ H ₅ O ₂ ⁻	85.039			3.02	0.18
S ⁻	31.972	NaSS	Background level	Background level	0.26
SO ⁻	47.969				0.24
SO ₃ ⁻	79.970				14.74
C ₆ H ₄ SO ₃ ⁻	156.001				1.43
C ₆ H ₆ SO ₃ ⁻	158.021				0.10
C ₇ H ₆ SO ₃ ⁻	170.020				0.73
C ₆ H ₆ SO ₃ Na ⁻	181.012				0.98
C ₈ H ₆ SO ₃ ⁻	182.020				0.27
C ₈ H ₇ SO ₃ ⁻	183.030				8.00
C ₈ H ₈ SO ₃ ⁻	184.035				0.91
C ₈ H ₉ SO ₃ ⁻	185.032				0.62
C ₈ H ₁₀ SO ₃ ⁻	186.043				0.13
C ₇ H ₇ SO ₃ Na ⁻	194.020				0.06
C ₁₂ H ₃ O ₃ ⁻	195.025				0.33
C ₈ H ₆ SO ₃ Na ⁻	208.032				0.05
C ₁₄ H ₉ O ₂ ⁻	209.037				0.32
C ₈ H ₁₀ SO ₃ -C ₄ H ₅ O ₂	271.046				0.10
C ₈ H ₁₀ SO ₃ -C ₂ H ₃ O ₂ -C ₃ H ₄	285.065				0.06
C ₈ H ₁₀ SO ₃ -C ₂ H ₃ O ₂ -C ₃ H ₆	287.071				0.05
C ₁₅ H ₁₃ S ₂ O ₆ ⁻	353.080				0.07

Then, the I_0 values of Ti2p and N1s for a clean titanium nitride surface were calculated by substituting $d_{\text{Carbon}} = 1.3$ nm with λ_{Ti} or λ_{N} into Eq. (2). Finally, the thickness of the MPS layer could be determined using Eq. (2). Since these measurements were made in the hybrid mode (large photoelectron acceptance angle), an effective photoelectron take-off angle of $\theta = 40^\circ$ was used for this calculation.⁴⁹ Now that values for I (the measured Ti2p or N1s intensity from the MPS modified titanium nitride), I_0 (the calculated Ti2p or N1s intensity for a clean titanium nitride surface), λ and θ ($=40^\circ$) are known, the estimated XPS thicknesses were calculated to be 1.4 nm with λ_{Ti} and 1.7 nm with λ_{N} . These values are consistent with the presence of a monolayer of MPS molecules.

The high-resolution Ti2p peak from the MPS modified titanium nitride [Table II and Fig. 2(b)] exhibits the same three components at 455.3, 456.8, and 458.3 eV as the bare titanium nitride. The TiN peak at 455.3 eV is smaller, and the titanium oxynitride and titanium dioxide peaks at 456.8 eV and 458.3 eV are slightly higher when comparing the bare titanium nitride sample to the MPS modified titanium nitride sample. The titanium oxynitride and titanium dioxide are commonly found on the surface of titanium nitride.³¹ The increase of these components on the MPS modified titanium nitride could result from the homogeneity and uniformity of the MPS film, leading to a smaller sampling depth into the substrate. On the other hand, the larger inelastic background on the high binding energy side of the Ti2p peak observed in the spectrum of the bare titanium nitride [Fig. 2(a)] would suggest a thicker layer of adventitious carbon.^{51–53} This discrepancy could be explained by the fact that the adventitious carbon layer is thick and nonuniform.

The high resolution C1s spectrum of the MPS modified titanium nitride sample [Table II and Fig. 2(e)] shows a decrease in the 285.0 eV component as compared to the bare titanium nitride. At the same time, the increase of the two components at 286.5 eV and 288.9 eV is attributed to the methacrylate groups presented in the MPS molecules. The high-resolution O1s XPS peak from the MPS modified titanium nitride [Table II and Fig. 2(h)], was fit with three components at 530.2 eV (TiO_2), 531.8 eV (titanium oxynitride plus the carbonyl oxygen in MPS), and 533.1 eV (the ester oxygen in MPS). Since the MPS oxygen signal overlapped with the titanium oxynitride peak in the O1s spectrum, it is difficult to quantify the MPS presence directly from the O1s spectra, and therefore, complementary analysis with ToF-SIMS was done (Tables III and IV, Figs. 4 and 5). ToF-SIMS has higher sensitivity (ppm level) and it provides specific molecular information. The main ToF-SIMS fragments detected from the MPS modified titanium surface were silicon containing fragments (SiO_xCH_y ions) originating from the MPS trimethoxysilane tail group and $\text{C}_x\text{H}_y\text{O}_z$ ions, which are unique to the molecular structure of the MPS methacrylate head group (Figs. 4 and 5). These oxygen-containing fragments could be easily distinguished from the oxynitride characteristic fragments (TiO_xN_y). In addition, small $\text{TiSiO}_x\text{CH}_y$ fragments were detected, which are consistent with covalent bonding

between the MPS methoxysilane tails and the titanium oxynitride surface. Thus, these results suggest that the MPS molecules were successfully attached onto the titanium nitride surface, displacing the adventitious contamination and forming a thin uniform film.

The pNaSS film was grafted from the MPS modified titanium nitride through free radical polymerization.^{7,24–27} The XPS surface elemental composition of pNaSS grafted surface [Table I and Fig. 2(c)] showed the presence of sulfur and sodium, which are unique to the NaSS molecules, providing evidence of the successful grafting of pNaSS from the MPS modified surface. XPS also exhibited an increased carbon signal as well as decreased titanium and nitrogen signals as compared to the MPS modified surface. This is consistent with the fact that pNaSS contains mainly carbon and forms an overlayer film on top of the MPS modified surface. Similar to the MPS layer, the thickness of the organic layer (MPS + pNaSS) on the pNaSS grafted titanium nitride was estimated by the attenuation of the Ti2p and N1s signal intensities employing Eqs. (2) and (3). Based on these equations, the estimated thickness of the MPS-NaSS layer is ~ 3.2 nm (estimated thicknesses of 2.9 nm calculated with λ_{Ti} and 3.4 nm calculated with λ_{N}). Subtracting the MPS layer thickness (see above) from the total film thickness, results in a pNaSS layer thickness of ~ 1.7 nm. This film thickness is consistent with the grafting of several NaSS monomer units. The number of NaSS monomer units grafted into a film of this thickness will depend on the exact tilt and twist angles of the polymer backbone and side chains. For a 1.7 nm film, this could range between 2 and 10 NaSS monomer units. Also, as the XPS measurements are done in an ultrahigh vacuum environment, the XPS determined film thickness represents the dry thickness of the MPS + pNaSS overlayer. It is likely the MPS + pNaSS film thickness will increase when the sample is placed in an aqueous environment.

The high-resolution C1s shows an increase in the hydrocarbon peak at 285.0 eV [Table II and Fig. 2(f)]. This peak accounts for $\sim 85\%$ of the carbon signal, consistent with the fact that seven of eight carbons in NaSS are hydrocarbon species. The high resolution XPS O1s spectrum shows an increase in the peak at 531.8 eV [Table II and Fig. 2(i)] due to the contribution of the S=O groups present in the NaSS film. Negative secondary ion fragments from the ToF-SIMS experiments (Table IV and Fig. 5) provide direct evidence for the presence of NaSS in the grafted films. Mainly SO_x and $\text{C}_y\text{H}_z\text{SO}_x$, but also $\text{C}_y\text{H}_z\text{SO}_x\text{Na}$, fragments are all directly related to the structure of NaSS group.²⁷ In particular, the strong intensity of $\text{C}_8\text{H}_7\text{SO}_3$ fragment (NaSS monomer) indicates the presence of NaSS on the titanium nitride surface. ToF-SIMS also detected fragments ($\text{C}_8\text{H}_{10}\text{SO}_3$, $\text{C}_4\text{H}_5\text{O}_2$ and $\text{C}_8\text{H}_{10}\text{SO}_3$ - $\text{C}_2\text{H}_3\text{O}_2$ - C_3H_2) that were consistent with covalent attachment of NaSS to MPS. In addition, the fragment originating from two NaSS units ($\text{C}_{15}\text{H}_{13}\text{S}_2\text{O}_6$) was detected, consistent with polymerization of the NaSS groups in the grafted film. The ToF-SIMS intensities of the titanium containing fragments and the MPS fragments

decreased after pNaSS grafting (Tables III and IV). Similar to the XPS analysis, this observation is explained by the fact that the pNaSS film forms an overlayer film on top of the MPS modified titanium surface.

Toluidine blue colorimetric assay was done to estimate the surface density of the NaSS groups. The amount of grafted polymer, G , was first calculated from the assay based on Eq. (1) and then divided by the surface area of the sample. The projected sample area was corrected based on the AFM analysis (Fig. 1) to estimate the actual surface area. The surface density of the pNaSS was estimated as 260 ± 70 ng/cm². Finally, preliminary radiolabeling experiments of protein adsorption have shown that ca. 200 ng/cm² of bovine serum albumin (BSA) adsorbed onto the bare titanium nitride surface and ca. 300 ng/cm² of BSA adsorbed onto the pNaSS grafted titanium nitride. This 50% increase in BSA adsorption on the pNaSS grafted surface compared to the bare surface is another indication of the successful biomodification of the titanium nitride surface with pNaSS.

IV. CONCLUSIONS

A two-step procedure was developed to graft bioactive pNaSS thin films from titanium nitride surfaces. MPS molecules were used as a cross linker between the titanium nitride surface and the pNaSS film. After attaching the MPS molecules onto the titanium nitride surfaces, XPS and ToF SIMS data indicated that the titanium nitride substrates were covered by the MPS films with a thickness of ~ 1.5 nm. ToF-SIMS detected fragments from MPS and MPS plus titanium. After pNaSS grafting, the XPS composition showed the appearance of sulfur and sodium and an increase of the carbon to titanium ratio. The pNaSS film thickness was estimated from the XPS analysis as ~ 1.7 nm. ToF-SIMS detected fragments from NaSS in the grafted film along with a decrease in titanium containing fragments. Finally, toluidine blue colorimetric assay showed the surface density of the pNaSS groups was 260 ± 70 ng/cm², while preliminary BSA adsorption experiments have shown a 50% increase in the adsorption on the pNaSS grafted titanium nitride surfaces.

ACKNOWLEDGMENT

This research was funded by NIH Grant EB-002027 to the National ESCA and Surface Analysis Center for Biomedical Problems.

- ¹S. Piscanec, L. C. Ciacchi, E. Vesselli, G. Comelli, O. Sbaizero, S. Meriani, and A. De Vita, *Acta Mater.* **52**, 1237 (2004).
- ²U. Türkan, O. Öztürk, and A. E. Eroğlu, *Surf. Coat. Technol.* **200**, 5020 (2006).
- ³B. Subramanian, R. Ananthakumar, and M. Jayachandran, *Surf. Coat. Technol.* **205**, 3485 (2011).
- ⁴M. Gradzka-Dahlke, A. Bukrym, E. Chojńska, Z. Gosiewski, and Z. Kulesza, *Solid State Phenom.* **147–149**, 782 (2009).
- ⁵Y. M. Ko and H. C. Choe, *Mater. Sci. Forum* **539–543**, 726 (2007).
- ⁶M. Windler and R. Klabunde, *Titanium for Hip and Knee Prostheses in Titanium in Medicine*, edited by D. M. Brunette, P. Tengvall, M. Textor, and P. Thomsen (Springer-Verlag, Berlin, Heidelberg, 2001), pp. 703–746.

- ⁷G. Helary, F. Noirclere, J. Mayingi, and V. Migonney, *Acta Biomater.* **5**, 124 (2009).
- ⁸G. Zorn, I. Gotman, E. Y. Gutmanas, R. Adadi, and C. N. Sukenik, *J. Mater. Sci.: Mater. Med.* **18**, 1309 (2007).
- ⁹T. W. Eckenhoff and T. Pintauer, *Inorg. Chem.* **46**, 5844 (2007).
- ¹⁰K. Matyjaszewski, H. Dong, W. Jakubowski, J. Pietrasik, and A. Kusomo, *Langmuir* **23**, 4528 (2007).
- ¹¹J. K. Oh, K. Min, and K. Matyjaszewski, *Macromolecules* **39**, 3161 (2006).
- ¹²X. Fan, L. Lin, J. L. Dalsin, and P. B. Messersmith, *J. Am. Chem. Soc.* **127**, 15843 (2005).
- ¹³B. Barthélémy, S. Devillers, I. Minet, J. Delhalle, and Z. Mekhalis, *J. Colloid Interface Sci.* **354**, 873 (2011).
- ¹⁴X. Fan, L. Lin, and P. B. Messersmith, *Biomacromolecules* **7**, 2443 (2006).
- ¹⁵S. M. Kang, B. S. Lee, W.-J. Kim, I. S. Choi, M. Kil, H.-J. Jung, and E. Oh, *Macromol. Res.* **17**, 174 (2009).
- ¹⁶F. Zhang, F. J. Xu, E. T. Kang, and K. G. Neoh, *Ind. Eng. Chem. Res.* **45**, 3067 (2006).
- ¹⁷R. N. Foster, A. J. Keefe, S. Jiang, and D. G. Castner, *J. Vac. Sci. Technol., A* **31**, 06F103 (2013).
- ¹⁸B. Hojjati and P. A. Charpentier, *J. Polym. Sci., Part A: Polym. Chem.* **46**, 3926 (2008).
- ¹⁹B. Hojjati, R. Sui, and P. A. Charpentier, *Polymer* **48**, 5850 (2007).
- ²⁰C. Li and B. C. Benicewicz, *Macromolecules* **38**, 5929 (2005).
- ²¹L.-P. Wang, Y.-P. Wang, K. Yuan, and Z.-Q. Lei, *Polym. Adv. Technol.* **19**, 285 (2008).
- ²²K. Yuan, Z.-F. Li, L.-L. Lü, and X.-N. Shi, *Mater. Lett.* **61**, 2033 (2007).
- ²³A. Michiardi, G. Hélar, P. -C. T. Nguyen, L. J. Gamble, F. Anagnostou, D. G. Castner, and V. Migonney, *Acta Biomater.* **6**, 667 (2010).
- ²⁴G. Pavon-Djavid, L. J. Gamble, M. Ciobanu, V. Gueguen, D. G. Castner, and V. Migonney, *Biomacromolecules* **8**, 3317 (2007).
- ²⁵M. Ciobanu, A. Siove, V. Gueguen, L. J. Gamble, D. G. Castner, and V. Migonney, *Biomacromolecules* **7**, 755 (2006).
- ²⁶C. Vaquette, V. Viateau, S. Guérard, F. Anagnostou, M. Manassero, D. G. Castner, and V. Migonney, *Biomaterials* **34**, 7048 (2013).
- ²⁷G. Zorn, J. E. Baio T. Weidner, V. Migonney, and D. G. Castner, *Langmuir* **27**, 13104 (2011).
- ²⁸E. S. Gawalt, M. J. Avaltroni, N. Koch, and J. Schwartz, *Langmuir* **17**, 5736 (2001).
- ²⁹G. Zorn, I. Gotman, E. Y. Gutmanas, R. Adadi, G. Salitra, and C. N. Sukenik, *Chem. Mater.* **17**, 4218 (2005).
- ³⁰R. Adadi, G. Zorn, R. Brener, I. Gotman, E. Y. Gutmanas, and C. N. Sukenik, *Thin Solid Films* **518**, 1966 (2010).
- ³¹G. Zorn, R. Adadi, R. Brener, V. A. Yakovlev, I. Gotman, E. Y. Gutmanas, and C. N. Sukenik, *Chem. Mater.* **20**, 5368 (2008).
- ³²I. Bertóti, M. Mohai, J. L. Sullivan, and S. O. Saied, *Appl. Surf. Sci.* **84**, 357 (1995).
- ³³N. C. Saha and H. G. Tompkins, *J. Appl. Surf. Phys.* **72**, 3072 (1992).
- ³⁴K. Uetani, H. Kajima, A. Takagi, I. Tokomoto, Y. Koizumi, and K. Nose, *Mater. Trans.* **42**, 403 (2001).
- ³⁵J. Schwartz, M. J. Avaltroni, M. P. Danahy, B. M. Silverman, E. L. Hanson, J. E. Schwarzbauer, K. S. Midwood, and E. S. Gawalt, *Mater. Sci. Eng., C* **23**, 395 (2003).
- ³⁶A. Hucknall, D.-H. Kim, S. Rangarajan, R. T. Hill, W. M. Reichert, and A. Chilkoti, *Adv. Mater.* **21**, 1968 (2009).
- ³⁷B. M. Silverman, K. A. Wiegand, and J. Schwartz, *Langmuir* **21**, 225 (2005).
- ³⁸A. Ulman, *Chem. Rev.* **96**, 1533 (1996).
- ³⁹J. R. Hull, J. J. Shannon, G. S. Tamura, and D. G. Castner, *Biointerphases* **2**, 64 (2007).
- ⁴⁰C. P. Tripp and M. L. Hair, *Langmuir* **8**, 1120 (1992).
- ⁴¹J. D. Le Grange, J. L. Markham, and C. R. Kurjian, *Langmuir* **9**, 1749 (1993).
- ⁴²R. Maoz and J. Sagiv, *J. Colloid Interface Sci.* **100**, 465 (1984).
- ⁴³J. Gun and J. Sagiv, *J. Colloid Interface Sci.* **112**, 457 (1986).
- ⁴⁴J. Gun, R. Iscovici, and J. Sagiv, *J. Colloid Interface Sci.* **101**, 201 (1984).
- ⁴⁵N. Tillman, A. Ulman, J. S. Schildkraut, and T. L. Penner, *J. Am. Chem. Soc.* **110**, 6136 (1988).

- ⁴⁶B. D. Ratner and D. G. Castner, *Electron Spectroscopy for Chemical Analysis in Surface Analysis The Principle Techniques*, 2nd ed., edited by J. C. Vickerman and I. S. Gilmore (Wiley, New York, 2009), pp. 47–102.
- ⁴⁷S. Tanuma, C. J. Powell, and D. R. Penn, *Surf. Interface Anal.* **20**, 77 (1993).
- ⁴⁸J. E. Castle, *J. Electron Spectrosc. Relat. Phenom.* **178–179**, 347 (2010).
- ⁴⁹S. D. Techane, L. J. Gamble, and D. G. Castner, *J. Phys. Chem. C* **115**, 9432 (2011).
- ⁵⁰S. Tanuma, C. J. Powell, and D. R. Penn, *Surf. Interface Anal.* **21**, 165 (1994).
- ⁵¹H. S. Hansen, S. Tougaard, and H. Biebuyck, *J. Electron Spectrosc. Relat. Phenom.* **58**, 141 (1992).
- ⁵²S. Tougaard, *Appl. Surf. Sci.* **100–101**, 1 (1996).
- ⁵³J. E. Castle, H. Chapman-Kpodo, A. Proctor, and A. M. Salvi, *J. Electron. Spectrosc. Relat. Phenom.* **106**, 65 (2000).