Effect of Nitrogen and Oxygen Incorporated into TMSAA Plasma Coating on Surface-Bound Heparin Activity

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ABSTRACT: To investigate the influence of nitrogen and oxygen incorporated into *N*-trimethylsilylallyamine (TMSAA) plasma coating on heparin binding to the surfaces, four types of monomer combinations were utilized. Those combinations include TMSAA alone, TMSAA mixed with nitrogen (TMSAA + N_2), with air (TMSAA + air), and with oxygen (TMSAA + O_2). Fourier transform infrared (FTIR) spectroscopy was employed to study the coating of the bulk structure. The thickness and surface morphology of the coatings were measured using atomic force microscopy (AFM). Electron spectroscopy for chemical analysis (ESCA) and the contact angle were used to investigate the surface elemental composition and hydrophilicity, respectively. It was found that the incorporation of oxygen into the coating formation significantly increased the deposition rate of the TMSAA + O_2 coating, but the heparin activity was the least even though it made the coating surface more hydrophilic. This is considered to have resulted from the loss of nitrogen in the coating structure due to the oxygen replacement to nitrogen. The nitrogen incorporated into the coating had no noticeable effect on the heparin surface-binding ability. The TMSAA + air plasma grafting process exhibited the best heparin attachment to the surface, which could be largely attributed to its highest surface roughness, although the nitrogen composition was decreased to some extent compared to the pure TMSAA plasma coating. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 1875–1883, 2003

Key words: plasma polymerization; FT-IR; ESCA / XPS; atomic force microscopy (AFM)

INTRODUCTION

In recent years, the plasma polymerization process has been widely used in applications very effectively, many of them being used in the preparation of biomedical materials with unique performance and the manufacturing of medical devices.[1] Heparin is an anticoagulant that inhibits blood clotting and is widely used for the treatment and prevention of thromboembolic disorders, reducing the risk of clots traveling and obstructing blood flow to vital organs (e.g., the heart and lungs), causing thrombosis or stroke. Heparin exerts its anticoagulant activity by catalyzing clotting protease inhibition, particularly that of thrombin by antithrombin (AT) and the heparin cofactor II.[2] Heparin activity is one of the important properties related to the biomedical application of materials including alloys and synthetic polymers, such as stainless steel (SS), nitinol, polyurethane, polyethylene, polypropylene, and polytetrafluoroethylene, which have been widely used for the manufacturing of medical devices like guide wires, stents, catheters, and sutures. A number of studies have been devoted to ways of modifying the surface properties of materials to make them more thromboresistant. One way of doing this is to bind heparin to the surface.[3–6] To strengthen heparin attachment to biomaterial surfaces, a couple of years ago, a proprietary multilayer coating technique (TRC®4.0) including plasma polymerization and dip chemical coating was developed at BioSurface Engineering Technologies (BioSET), Inc.[7] TRC®4.0 is a lubricious, biocompatible heparin coating, wherein sodium heparin is chemically conjugated to an underlying siloxane coating[8] through an amine-containing *N*-trimethylsilylallyamine (TMSAA) grafting layer. The siloxane coating and TMSAA grafting were carried out in a glow-discharge plasma reactor, whereas the heparin conjugation was performed through a wet chemical coating process. Amine functional groups have been considered to play an important role in heparin conjugation to biomaterial surfaces.[9–12] Other researchers also reported that amine-functionalized thin films prepared by radio-frequency plasma polymerization were used for subsequent grafting reactions or for direct biomedical applications usually involving exposure of the films to a solvent environment.[13] In the work of Dai et al., ammonia plasma was employed to equip the surfaces of a normally unreactive polymeric substrate with a sublayer of amine groups capable of reacting with polysaccharides in an aqueous solution.[14] In the present study, to improve the covalent binding of active heparin to the surface, which was measured by a kinetic heparin assay kit of Coatest®, the TMSAA plasma grafting in the multi-

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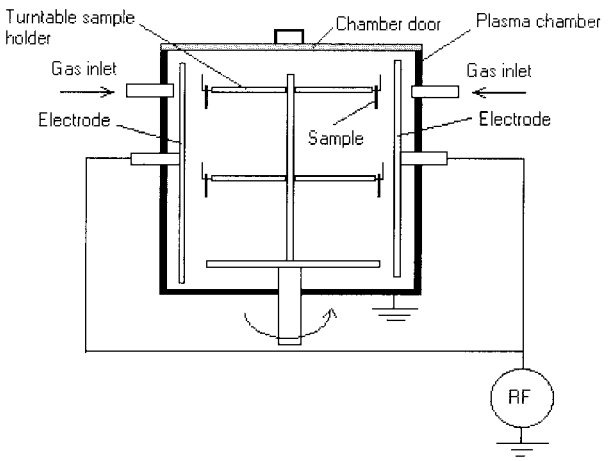

Figure 1 Plasma deposition system used in this study.

coating process was modified using a mixture of TMSAA with O_2, N_2, and air. The effect of N and O incorporation into the sublayer on the surface property was investigated using a variety of analysis methods, including FTIR, AFM, ESCA, and the contact angle. The heparin assay used in this study is based on the inactivation of thrombin by antithrombin III, so this type of assay provides an estimate of the amount of active heparin bound to the sample surfaces—in other words, the biological activity of heparin, rather than the amount of mass or heparin bound, since the binding process may somewhat inactivate the heparin.

EXPERIMENTAL

Plasma reactor system and materials used

The TMCTS [$O_4Si_4(CH_3)_4H_4$] liquid monomer with a purity of 95% plus was supplied by Gelest, Inc. (Tullytown, PA). Ammonia (NH_3) with a concentration of 99.999%, electronic grade, was purchased from Air Products and Chemicals, Inc. (Allentown, PA). *N*-Trimethylsilylallylamine [TMSAA, $CH_2{=}CHCH_2NHSi(CH_3)_3$] was obtained from Gelest, Inc., with purity of 95.8%, NMR grade. N_2 and O_2 gases were supplied by Air Products and Chemicals, Inc., with purities of 99.7 and 99.9%, industry grade, respectively.

The plasma deposition system used in this study is shown in Figure 1. This home-made plasma coating machine was designed and built by BioSET in 1999 in Salt Lake City, Utah. The glow discharge was ignited between the parallel-plate electrodes made of aluminum with a size of 7 × 7 in. The distance of the two electrodes was 6.5 in., and the electrodes were driven by 13.56 MHz power. The power supply RF10S was manufactured by RF Power Products, Inc. (Voorhees, NJ). The sample holder rotated at a speed of 60 rpm during all the plasma processes to assure the uniformity of the plasma treatment and plasma coating deposition. A base vacuum of higher than 10 mTorr was achieved in about 5 min using a turbomolecular pump (Osaka Vacuum, Model TS433, made in Japan), purchased from Osaka Vacuum, Ltd. (San Jose, CA) and a mechanical pump system consisting of a rotary vane vacuum pump (Alcatel 2033CP+) and a roots blower (Alcatel RSV 300B). The Alcatel pump system was procured from Alcatel Vacuum Products (Fremont, CA) and used as the fore-vacuum pump to the Osaka pump. The monomer or working gas was introduced to the plasma reactor through a gas mixer located at the outside of the reactor and two gas inlets at the two sides of the chamber. Then, the monomer or gas flowed into a gas distributor located in the front of the chamber to allow an even flow pattern to the discharge area between the two electrodes. All unreacted or nonpolymerizing species were pumped out from an exhaust port at the back of the chamber.

Plasma coating and wet chemical coating procedures of multilayer coating method

The typical plasma processes employed are listed in Table I, all of them consisting of three steps: NH_3/O_2 plasma etching or treatment, plasma coating of TMCTS, and TMSAA (or its mixture with N_2, O_2, or air) grafting. For the third step, the mass flow rate of TMSAA was kept constant at 42 standard cubic centimeters per minute (sccm) in all cases, and the mass flow rate of N_2, O_2, or air to the mixture was 16 sccm. The mass flow controller for TMSAA was a customized product from MKS Instruments, Inc. (Andover, MA). The mass flow rate of 42 sccm was utilized based on the optimization process that was done to obtain a quality coating without powder formation at a certain plasma discharge condition. Therefore, the molar ratio of TMSAA to N_2, O_2, or air has the same value of 2.6:1. The reason for keeping the TMSAA mass flow rate at a certain value is simply to see how those additional N_2 or O_2 molecules affect the plasma grafting process. Between any two successive steps, the plasma reactor was pumped down to 10 mTorr to minimize the possible residual effect from the prior step on the plasma process of the next step. All the plasma coating processes were performed in an automation mode, which was controlled by a computer to eliminate the variation between runs usually encountered with manually operating plasma processes. All the plasma parameters used in the three steps, that is, the mass flow rate and molar ratio of the monomer mixtures, the pressure, the discharge power and time, and even the base vacuum for each step, had been preset before starting a run.

After the plasma coating, all the samples went through a chemical dip-coating process to make the sodium heparin covalently bind to the plasma-coated sample surfaces. All those samples were made of 316 SS with a size of 7.1 × 7.1 × 2 mm. BioSET has

TABLE I
Description of Plasma Process Used in the Study

	Item			
	1	2	3	4
	Process ID			
	TMSAA	TMSAA + N_2	TMSAA + air	TMSAA + O_2
Step 1. etching	$NH_3 + O_2$	$NH_3 + O_2$	$NH_3 + O_2$	$NH_3 + O_2$
Flow rate (sccm)	40 (NH_3) + 10(O_2)	40 (NH_3) + 10(O_2)	40 (NH_3) + 10(O_2)	40 (NH_3) + 10(O_2)
Pressure (m Torr)	50	50	50	50
Power (W)	110	110	110	110
Time (s)	420	420	420	420
Step 2: coating	TMCTS	TMCTS	TMCTS	TMCTS
Flow rate (sccm)	84	84	84	84
Pressure (m Torr)	55	55	55	55
Power (W)	83	83	83	83
Time (s)	4	4	4	4
Step 3: grafting	TMSAA	TMSAA + N_2	TMSAA + air	TMSAA + O_2
Flow rate (sccm)	42	42 (TMSAA) + 16(N_2)	42 (TMSAA) + 16(Air)	42 (TMSAA) + 16(O_2)
Pressure (m Torr)	65	65	65	65
Power (W)	35	35	35	35
Time (s)	480	480	480	480

developed a bifunctional poly(ethylene oxide) tether, polyoxyethylene bis(*N*-hydroxybenzotriazolyl)carbonate (HPEO).[15] HPEO will form urethane bonds with surface-based amino groups of the TMSAA coating and with the amino groups of aminoglycan sodium heparin. After the plasma coating process, those SS samples were placed in a 5% HPEO solution in CH_2Cl_2 for 10 min with occasional agitation during that period. Then, those samples were washed twice in the CH_2Cl_2 solution. At the end of the solvent rinse, the HPEO-coated samples were immersed into a 5% solution of sodium heparin in CH_2Cl_2 with a pH value at 8.5 for 20 min. Then, they were rinsed with running water (deionized) for 20 min. Finally, all the samples were dried in an oven at the temperature of 50°C for 30 min; then, all the samples were ready for measurement of the surface-bound heparin activity.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR is an extremely powerful analytical technique for both qualitative and quantitative analysis on coating the bulk property by associating definite characteristic absorption bands and their intensities with certain functional groups in the sample. The FTIR spectra were measured with an FTS-135 spectrometer, a product of Bio-Rad Laboratories, Inc. (Cambridge, MA), which was controlled by the Win-IR Foundation application software. The resolution used in the measurement was 4 cm^{-1}. The samples used for FTIR analysis were Si wafers, and quality FTIR spectra can be easily obtained for the TMSSA-series coatings. The FTIR spectra were analyzed to determine if there are any new functional groups containing nitrogen and/or oxygen formed in the TMSAA-series coatings.

Atomic force microscopy (AFM) analysis

AFM was performed to determine the surface morphology and coating thickness on a Si wafer. The AFM equipment used for this study was a TopoMetrix Explorer, located at the Chemistry Department, University of Utah. The probe type used was a Si_3N_4 tip with a typical radius of curvature of ~200 Å. The image size was 50 × 50 μm, and the scan rate was 75 μm/s. To facilitate the measurement, prior to the coating process, one part of the Si wafer surface was covered with normal Scotch™ tape and the rest of the surface was left open for plasma coating. After the coating was done, the tape was pulled off, and a clear boundary was formed between the coated and uncoated surfaces, and the coating thickness could be obtained by analyzing the scanned AFM image of the cross-section height profiles.

Electron spectroscopy for chemical analysis (ESCA)

ESCA analysis of the Si wafers coated with the plasma coating provides information of the elemental composition and chemical bonding states of the elements contained. ESCA was performed with a VG Scientific 220i-XL instrument equipped with a monochromatic

Al Kα X-ray (1486.7 eV) source at the Chemistry Department, University of Utah. Typical operating conditions for the X-ray source were a 400-μm nominal X-ray spot size (fwhm) operating at 15 kV, a 8.9 mA setting, and 100 W. The take-off angle was fixed at 90°. Survey spectra, from a 0 to 1200 eV binding energy, were collected at a 100 eV pass energy with an energy resolution of ~1.0 eV, a dwell time of 100 ms, and a total of two scans averaged. Quantification of ESCA was performed by area fitting under each component peak and using atomic sensitivity factors. The change in the elemental composition of nitrogen and oxygen, as well as of carbon and silicone, at the TMSAA-series coating surfaces was studied and correlated to the surface-bound heparin activity.

Contact-angle measurement

The static water contact angle was determined at room temperature. The contact angle formed between a sessile drop and its supporting surface is directly related to the forces at the liquid/solid interface, indicating the hydrophilic or hydrophobic characteristics of the surface. The measurement was performed with an NRL 100-00 contact-angle goniometer made by Ramé-hart, Inc. (Mountain Lakes, NJ) and distilled and deionized water was used for the drops. The contact-angle data will give information about the surface hydrophilicity change resulting from nitrogen and oxygen incorporation into the coatings.

Heparin activity assay

There are several ways to estimate the surface-bound heparin activity. A report from Chandler et al. showed that a kinetic assay based on the heparin-accelerated inactivation of thrombin by antithrombin III can be used to estimate the amount of active heparin bonds to the substrate surface.[16] Conventional heparin assays measure the anticoagulant effect of heparin in a clotting-time system. Such assays reflect the accelerated inactivation of clotting factors induced by heparin. The Coatest® heparin assay kit, a product from Chromogenix, Inc. (Milan, Italy, distributed by the Diapharma Group, Inc., West Chester, OH), was used to determine the amount of active heparin bound to the plasma polymer-coated samples. The heparin assay is based upon the inhibition of Factor Xa activity by antithrombin III (AT-III), which was activated via prior complexation with heparin.[17] The remaining amount of FXa in the test solution hydrolyses the chromogenic substrate S-2222, thus liberating the chromophoric group. The absorbance of the group, measured at 405 nm using a Shimadzu UV-vis recording spectrometer, UV160U, purchased from Shimadzu Scientific Instruments, Inc. (Columbia, MD), decreased linearly with an increasing concentration of heparin. The concentration of heparin was calculated from the standard curve prepared from human normal plasma with a known heparin concentration.

RESULTS AND DISCUSSION

The plasma etching process was operated at the condition of 110 W, 55 mTorr, and a total mass flow of 50 sccm for the gas mixture of NH_3/O_2 with a molar ratio of 4 to 1 for 420 s. The plasma treatment was considered to provide a clean and reproducible starting condition for further plasma polymer deposition to form a well-controlled surface layer.[18] Specifically, it was used to introduce amino functional groups and oxygen-containing groups[19–22] on the SS substrate surface for covalent chemical binding to the TMCTS coating. NH_3/O_2 plasma treatment on SS could also be directly used for biomedical application, such as for reducing platelet and leukocyte attachment to the surface.[22] The bright glow discharge in the vicinity of the two electrodes was seen with the naked eye through the view port on the top of the plasma reactor.

Plasma deposition of TMCTS is a low-energy process that deposits a thin layer of siloxane coating without altering the bulk properties of the substrate. The glow discharge in TMCTS was dark and no bright glow was seen, even though the SS surface was still modified, and the coating thickness for 4-s deposition was estimated to be 10–20 Å using extrapolation of the thickness data obtained from the AFM. The reason for this darkness is that the discharge was operated at a relatively low power, and it was chosen through an optimization process to minimize powder formation in the plasma of TMCTS. Actually, when the discharge power was set at 125 W, which is about 50% higher than the power level used in this study, a bright glow discharge can be easily seen with the naked eye. But, at the same time, a considerable amount of powder was seen in the vicinity of the electrodes close to the outlet of the plasma reactor. The contact angle of the SS chip coated with TMCTS for 4 s was 100°, a significant increase from 44° of the control chips without any plasma treatment or coating, indicating a considerable surface property change on the metal surface. This siloxane coating can be applied to many substrates other than SS, including polyethylene, polycarbonate, polypropylene, and polyurethane.[8]

To introduce amino functional groups to the surface of the TMCTS plasma coating so that heparin and other biomolecules can be tethered to the surface using poly(ethylene glycol), TMSAA was used as the monomer for the plasma grafting of stable, hydrolysis-resistant primary amino groups. This is also a low-energy plasma process, which was considered to minimize the loss of substrate bulk properties. Furthermore, the addition of N_2 and/or O_2 to the TMSAA monomer was carried out, since incor-

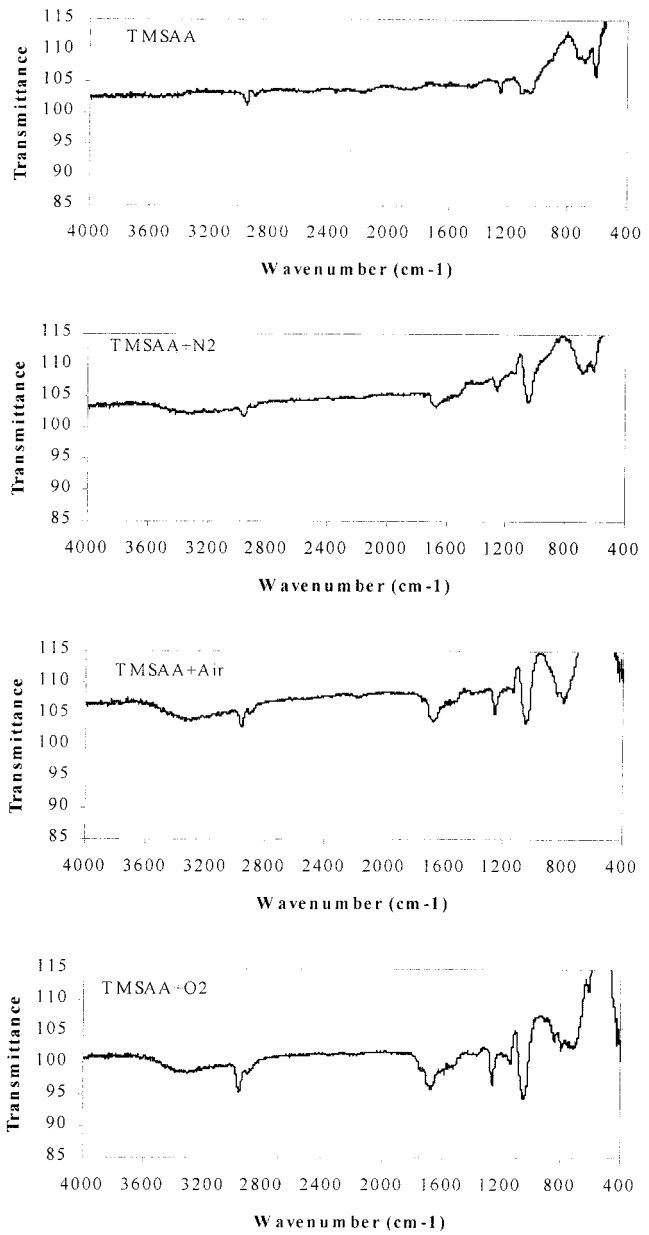

Figure 2 FTIR spectra of the plasma coatings.

poration of N_2 or O_2 has been recognized as a way to add some unique characteristic features to plasma polymerization.[23]

Bulk structure of TMSAA-based coatings

TMSAA has the following molecular structure:

$$CH_2{=}CH{-}CH_2{-}NH{-}Si(CH_3)_3$$

The chemical bond of N–Si in this structure is easy to break in the glow discharge to form reactive species. The difference of the absorption bands in the spectra is clearly shown in Figure 2. To facilitate comparison between those spectra, the same scale for the infrared beam transmittance was used for all the spectra. The assignments of the major absorption bands are tabulated in Table II.[24] The absorption bands at 2956, 2901, and 1254 cm^{-1} are present in all four spectra. For the TMSAA coating, the characteristic bands are 1050 and 662 cm^{-1}, which are attributed to the amine C–N stretching vibration (general range) and amine N—H deformation vibration (out-of-plane bending), respectively. In comparison with the TMSAA plasma coating, the two characteristic bands at 1050 and 662 cm^{-1} are higher in the coating made from TMSAA + N_2. As for the coatings of TMSAA + air and TMSAA + O_2, three new absorption bands appeared at 1668, 1140, and 842 cm^{-1} in the spectra compared to TMSAA and TMSAA + N_2. The absorption band at 1668 cm^{-1} was from the C═O stretching vibration in —CO—NH_3; the second one at 1140 cm^{-1} can be assigned to the Si—O stretching vibration in Si—O—CH_2. The band at 842 cm^{-1} occurring at TMSAA + air and TMSAA + O_2 can be assigned to Si—C stretching vibration in —$OSiCH_3$ (end group) because of the incorporation of oxygen into the film-formation process. This result indicates that oxygen participates in the coating formation process. The appearance of C═O in the TMSAA + N_2 coating was due mainly to oxygen incorporation during its exposure to air after the coating preparation.

Figure 3 shows the thickness of the coatings. It is easy to see that the thickness change is in the order TMSAA < TMSAA + N_2 < TMSAA + air < TMSAA + O_2. Because the mass flow rate of the TMSAA monomer, as well as other plasma parameters like pressure, discharge power, and time, was maintained at the same value in all cases, it is rational to say that

TABLE II
Assignments of Main Absorbance Bands in FTIR Spectra

Wave number (cm^{-1})	Assignments
2956	C—H asymmetric stretching of CH_3
2901	C—H symmetric stretching of CH_3
1668	C═O stretching in—CO—NH_2
1254	CH deformation in Si—CH_3
1140	Si—O stretching in Si—O—CH_2—
1050	Si—O stretching in Si—O—Si or amine C—N stretching
842	Si—C stretching in —$OSiCH_3$ (end group)
662	Amine N—H deformation

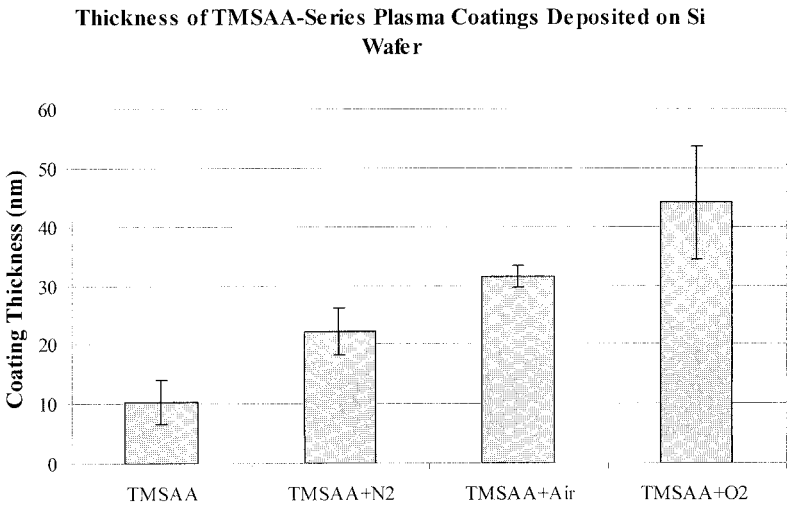

Figure 3 Thickness of plasma coatings of TMSAA, TMSAA + N_2, TMSAA + air, and TMSAA + O_2. (Data are mean ± standard deviation, $n = 3$.)

the input of N_2, air, or O_2 at a additional flow rate of 16 sccm into the plasma chamber positively enhanced the deposition rate by participating in the coating formation process. Furthermore, it seems that oxygen contributes most to the coating growth rate because of the high electronegativity of oxygen, making the incorporation of oxygen into the polymerization process easy. This trend is also reflected in the FTIR spectra of the four types of TMSAA-series plasma coatings, in which the transmittance at the wavenumber range of 2900–2960 cm^{-1} is lower from pure TMSAA to TMSAA + N_2, TMSAA + air, and TMSAA + O_2.

Surface characterization of plasma coatings

From the ESCA data shown in Figure 4 (the standard deviation was from three samples in each case), we can see that with addition of air to the TMSAA grafting process the atomic fraction of O was increased by about 77%. In contrast, the concentration of N and C was decreased by 34 and 14%, respectively. The Si composition in the coating was also increased, probably due to more TMSAA molecules being activated in the presence of air in the glow discharge. The ESCA data indicate that O atoms dominantly replace the position of C and N in the TMSAA-based plasma grafting process.

The contact angle of the plasma coatings on the Si wafer is shown Figure 5. The contact angle was measured about 1 month later after the plasma-coating process. All the samples were kept in plastic vials with caps tightly closed prior to the measurement. For comparison with the surface property of plasma coatings, the data for a bare Si wafer was also presented in the figure. As seen from the bar graph, the contact angle of the TMSAA coating surface is 78°, which is considered to be hydrophilic. However, with addition of N_2, O_2, or air (actually air is the mixture of N_2 and O_2 at a molar fraction of 77 and 20% respectively) to the TMSAA plasma grafting

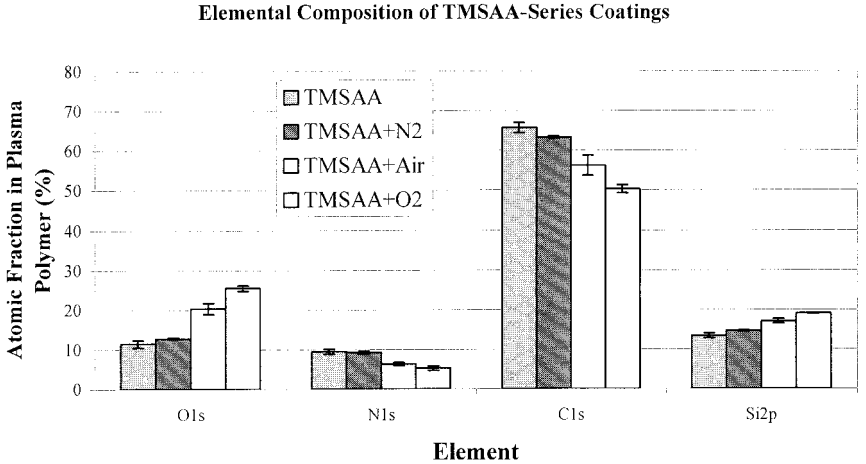

Figure 4 Atomic fraction of plasma coatings prepared at different conditions. (Data are mean ± standard deviation, $n = 3$.)

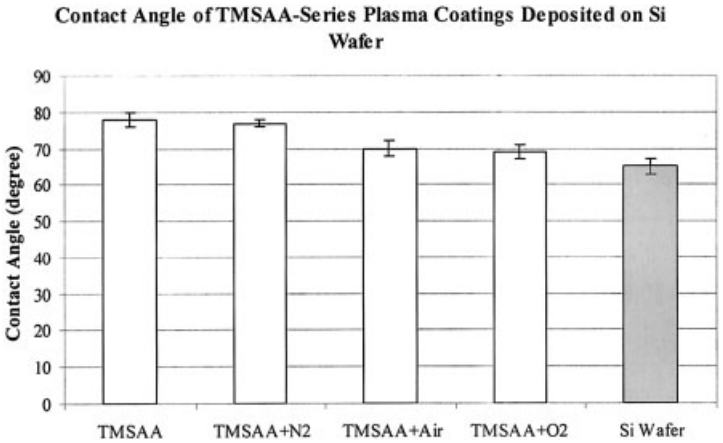

Figure 5 Contact angle of plasma coatings of TMSAA, TMSAA + N_2, TMSAA + air, and TMSAA + O_2. Substrate: Si wafer. (Data are mean ± standard deviation, $n = 3$.)

process, the surface activity of the consequently formed coating was modified, which was indicated by the decreasing contact-angle values. The increasing surface hydrophilicity of the coatings could make the heparin binding to the surface easier. However, the change in the water contact angle was not significant, which implies that this amount of oxygen incorporated into the TMSAA-based coatings may not contribute markedly to the surface hydrophilicity.

It seems that the heparin activity of the TMSAA-series coatings was increased by introducing air into the plasma grafting process (see Fig. 6). With introducing N_2 to the TMSAA monomer, the heparin activity was not changed considerably, compared to the pure TMSAA coating. The considerable drop in the heparin activity was found in the case of TMSAA + O_2, which has the highest coating thickness among the four types of coatings, indicating that the incorporation of more than enough oxygen into the coating is definitely detrimental to heparin binding to the surface. As seen in the ESCA data, since the N elemental composition at the coating surface was depleted by oxygen, the amine-containing functional group, which is considered responsible for heparin binding, in the coating became less.

Figure 7 shows surface roughness data of the four types of TMSAA-based samples, which were obtained by AFM. The roughness data were the algorithm mean values, which were calculated by the embedded software of the AFM equipment. The AFM images of the surfaces are shown in Figure 8. The surface of the TMSAA + air plasma coating renders the highest roughness among the four combinations, possibly due to the later kicking-out of the earlier incorporation of N atoms into a coating by the more reactive oxygen element formed in the discharge plasma. This high surface roughness was considered to contribute to the increase of heparin binding to the TMSAA + air coating surface.

CONCLUSIONS

Heparin activity is a critical property of biomaterials having widespread applications in medical devices. The proprietary multilayer coating technique of

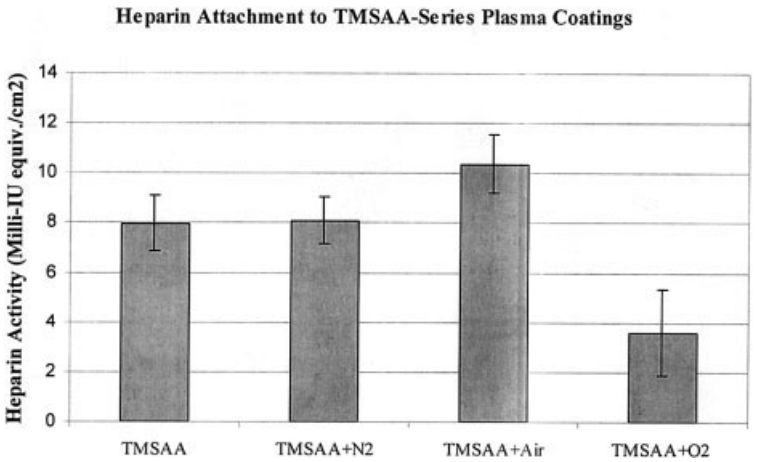

Figure 6 Surface-bound heparin activity of TMSAA-series plasma coatings. (Data are mean ± standard deviation, $n = 8$).

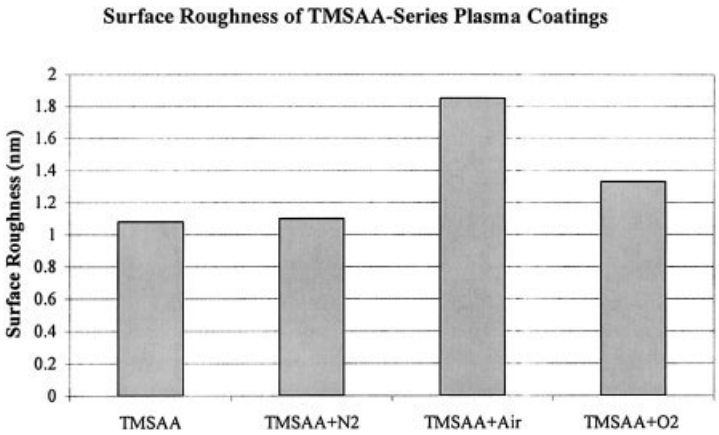

Figure 7 Surface roughness of TMSAA-series coatings.

TRC®4.0 developed by BioSET includes plasma coating and dip chemical coating processes to make sodium heparin covalently bind to biomaterial surfaces through a poly(ethylene glycol)-based tether, HPEO. The tether was considered to covalently bind both surface amino groups of the TMSAA coating and the amino groups of sodium heparin and to build a very stable heparin attachment to medical device surfaces. In this study, oxygen, nitrogen, and air were introduced into the TMSAA plasma grafting process at a certain mol fraction, and the result shows that active heparin binding to the plasma-coated SS chip surface was influenced by several factors. FTIR spectra indicated that, besides the presence of amino groups preserved from the TMSAA monomer molecules, the additional O and N from the gas mixture were incorporated into the plasma polymer in the form of functional groups like Si—O, C═O, C—N, etc. The thickness obtained by AFM shows that the incorporation of oxygen into the plasma polymer coating makes the deposition rate of the TMSAA + O_2 coating the highest, whereas the heparin activity of it was the

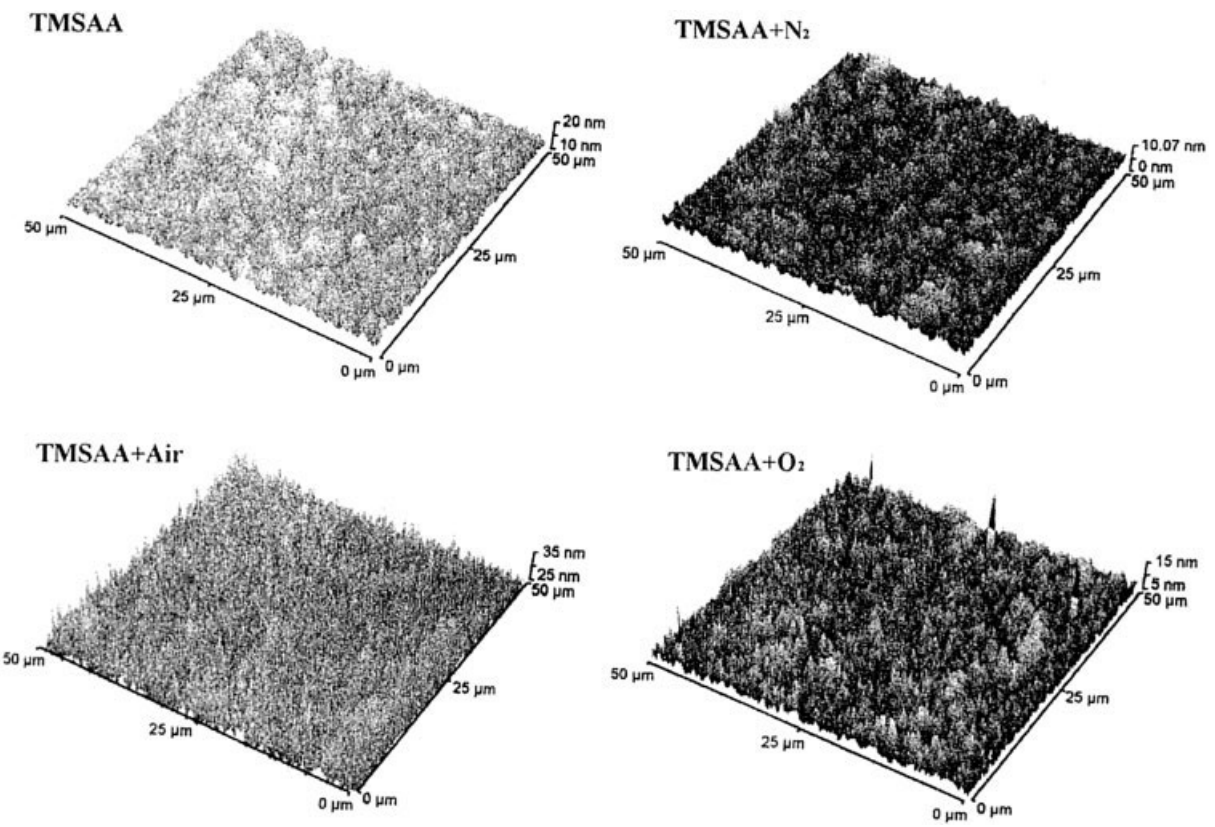

Figure 8 AFM images of plasma-deposited TMSAA-series coatings.

lowest. This may imply that more than enough oxygen incorporated into the coating surface was detrimental to heparin binding to the substrate surface; even the higher surface roughness of TMSAA + O_2 could ease this negative effect to a certain extent. The ESCA data also indicate that the incorporation of oxygen and nitrogen elements into the film-formation process have different effects on heparin activity. It seems that nitrogen in the TMSAA-based coatings has no tendency to increase heparin attachment. The contact angle of the coating surface was modified by the addition of O_2, N_2, or air to the TMSAA grafting process. Oxygen incorporation appears to slightly decrease the contact angle. This change in the surface hydrophilicity seems not to contribute considerably to heparin conjugation. It was found that the plasma grafting process of TMSAA mixed with air could achieve the best heparin attachment, most possibly due to the high surface roughness facilitating heparin binding to the substrate surfaces on the basis of no critical loss of nitrogen resulting from oxygen replacement.

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