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Plasma-Enhanced Generation of Stable PAA-and PVP-based Multi-layer Structures

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Summary

Oxygen/water vapor-plasma treated polished aluminum substrates were coated with poly(acrylic acid) (PAA) using deeping-coating technique, and subsequently heated. ESCA, chemical derivatization and SEM confirmed the successful coating of substrates. It was shown that the covalently attached PAA macromolecules exhibit anti-bacterial characteristics. Samples coated with PAA exposed to a 5 strain mixture of *Listeria monocytogenes* for 24 hours resulted in 98 % decrease in the bacterial population. A mix of three different bacteria, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Escherichia coli* was also tested. A reduction of 82 to 96 % of bacterial numbers was obtained. Experimental results indicated that double layer structures could also be prepared from PAA-coated surfaces. PAA and poly(vinylpyridine) (PVP) double layers were successfully generated.

Introduction

Thin organic polymer films and multi-layered assemblies have shown significant application potential as a novel class of materials for the generation controlled structure and thickness functional surface layers. Recently, polyelectrolyte multilayers became very attractive in particular as biomedical coatings. Specially designed polyelectrolyte layers can function as semi-permeable membranes or host bioactive molecules such as peptides proteins or various drugs with the preservation of their chemical and conformational structures, and activities [1-3]. It has been even shown that randomly arranged supermolecular species, such as viruses, incorporated in multilayer networks can reorganize into ordered structures at the surface [4].
Multilayers based on poly (acrylic acid) (PAA) and poly (4-vinylpyridine) (PVP) were recently investigated [5-8]. It was concluded from Fourier infrared analysis that the interaction between the two polymers is based on hydrogen bonding. Unfortunately, the stability of multilayer structures on various polymer or metal surfaces significantly depend on the adhesion of the first layer to the substrate, varying ionic strength and pH, and even on the level of the mechanical stress present during exploitation.

In this contribution a combined plasma-enhanced and conventional chemical approach is proposed for the attachment of PAA onto aluminum substrate surfaces followed by the subsequent built-up of PAA- and PVP-based layered antibacterial assemblies.

Experimental

Materials and Methods

PAA (MW 250 KDa; 5% water solution), and PVP (MW 60 KDa) were purchased from Aldrich Co. Mirror-polished 5052 aluminum sheets were obtained from McMaster-Carr Supply Company (Chicago, IL). The sheets were cut into 1 inch diameter disk-shaped coupons which were used as substrates.

Electron spectroscopy for chemical analysis (ESCA; Perkin-Elmer Physical Electronics 5400 small area ESCA system; Mg source; 15 kV; 300 W; take-off angle 45º; Perkin-Elmer, Palo Alto, CA) was used to characterize the surfaces of virgin and modified substrates. Carbon atomic concentrations were evaluated, and the binding energy (BE) values of carbon and nitrogen atoms located in nonequivalent positions (different chemical linkages) were analyzed. In order to correct for surface-charge-origin BE shifts, calibrations were performed based on the Au 4f5/2 (87.6 eV) and 4f7/2 (83.9 eV) BE values.

Plasma-induced changes of substrate surface morphologies were evaluated by electron microscopy using a LEO 1530 Field Emission, Digital Scanning Electron Microscope (LEO Electron Microscopy Inc.; Thornwood, NY).

Contact angle measurements were performed by the projected sessile droplet method with deionized water (1μL), with an OCA-15 Goniometer (Future Digital Scientific Co., Bethpage, NY).

Fluorescence labeling of H_2O/O_2 plasma-treated aluminum substrates for evidencing the presence of –OH functionalities was performed according to the following procedure: The substrates were immersed in 2.36 mg/mL 9-anthracene carboxylic acid/methanol solution for 15 minutes, followed by washing 3 times with 5 mL ether. The labeled samples were dried and then their fluorescence was measured using a Spex Fluorolog Tau-2 fluorescence spectrometer (excitation: 370 nm; 2.9 mm slits and 5 nm bandpass for both excitation and emission; Xe lamp-450W; double grating monochromator on excitation and emission).

Chemical derivatization of PAA-coated samples was done using toluidine blue O staining (TB) [9]. Samples were placed in a 0.5 mM TB solution for 6 h at 30°C, then were washed with 9 N sodium hydroxide solution three times. 50% acetic acid was used to remove the dye which was measured by a UV-visible spectrophotometer.

The aluminum surfaces were plasma treated in an oxygen/water vapor environment using a parallel plate, 45 kHz RF-plasma tool described earlier [10]. The following experimental parameters were used: Base pressure in the reactor: 50 mTorr; total pressure in the absence of plasma: 200 mTorr; H_2O/O_2 partial pressures 100/50 mTorr; RF-power dissipated: 100 W; plasma-exposure time: 120 seconds.

In a typical experiment seven aluminum substrates were positioned symmetrically on the lower, grounded electrode and exposed to the water/oxygen plasma for the selected treatment time. At the end of the plasma treatment the substrates were removed from the reactor, immersed in a 5 % water-based PAA solution for 1 hour, dried in the air and oven heated for 2 hours at 110°C, then kept in deionized (DI) water for 1 hour and rinsed 3 times using DI water.

In a separate experiment, the PAA-coated substrates were exposed to argon plasma for 1 minute under the following experimental conditions: power 50 W, pressure 150 mTorr. ESCA data (not shown) indicate the presence of plasma-induced surface decomposition reactions. Accordingly further experiments were performed only by using oxygen-plasma exposure of aluminum followed by heating process.
Part of the PAA-modified substrates were selected for analytical evaluation and part of them were consecutively coated with a PVP layer using a "deep-coating" technique (1 % w/w PVP / methanol solution; 1 hour immersion time) followed by washing with methanol for 5 times. After ultrasound cleaning (Sonicor, SC-101TH, Sonicor Instrument Corporation, Copiague, NY; PAA/water and PVP/methanol) for 10 min, the samples were analyzed by ESCA.
The antibacterial activity of PAAc-coated substrates was evaluated using a variety of bacteria. We tested *Listeria monocytogenes*, a foodborne pathogen, or a mixture of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*, the latter two being opportunistic pathogens. For *L. monocytogenes*, five strains were grown overnight at 37°C in trypticase soy broth with 1% yeast extract (TSB; Becton Dickinson Microbiology Systems, Cockeysville, MD), combined to form a five-strain mixture and diluted with sterile distilled water to achieve a bacterial concentration of about 6 log colony forming units (CFU)/mL. We have used this combination routinely in our studies to evaluate antifouling surfaces [10 – 13]. *E. coli*, *P. aeruginosa* and *S. epidermidis* were grown up individually overnight in TSB, diluted to achieve about 5 to 6 log CFU/mL and combined into a three-strain mixture. Control and PAA-coated substrates were soaked in sterile distilled water for 15 minutes, rinsed and air-dried in a petri dish. Each substrate was inoculated with 100 μL of the selected bacterial mixture and incubated at room temperature in a humidified chamber for 24 hours. After incubation, each substrate was added to a tube containing 10 mL 10 mM phosphate-buffered saline with 0.05% Tween 80 (pH 7.2) and glass beads. After 5 minutes, the substrate was swabbed with a sterile swab (Fisher Scientific) and vortexed to detach bacteria from the substrate surfaces. The number of viable bacteria was evaluated by plating onto brain heart infusion agar (Becton Dickinson) plates. The plates were incubated at 30C for 48 hours and the numbers of CFUs were counted and expressed as log CFU/coupon.

Results and discussions

Survey ESCA data collected from oxygen/water vapor plasma-exposed aluminum samples and plasma-treated samples subsequently coated with PAA and PVP are presented in Table 1. It can be observed that oxygen- and aluminum atoms are present in the oxygen and water vapor modified aluminum substrates, and that the relative surface atomic concentrations of PAA, PVP and PAA/PVP coated samples indicate the existence of carbon, oxygen and nitrogen atoms. The high resolution (HR) 72 eV binding energy (BE) peak of oxygen/H_2O-plasma treated aluminum substrate is attributed to the presence of aluminum atoms and the 74.6 eV peak is related to the existence of aluminum oxide and hydroxide in the surface layer (Figure 1).
It is interesting to note that the presence of –OH functionalities on the O_2/H_2O-plasma-treated substrates could not be evidenced by fluorescence labeling (Figure 2). Non-modified aluminum substrates and substrates washed using an ultra sound bath still exhibited high-intensity fluorescence after labeling them with 9-antracene

carboxylic acid, while unmodified and ultrasound-assisted washed, and subsequently O_2/H_2O-plasma-exposed substrates did not exhibit notable fluorescence (Figure 2).

Table 1. ESCA relative atomic composition of aluminum samples after plasma treatment and coating with various macromolecular layers.

Samples	C 1s %	O 1s %	Al 2p %	N 1s %
O_2+H_2O	23.1	43.1	33.8	-
PAA	53.2	39.6	7. 3	-
PVP	87.9	0	0	12.1
PAA+PVP	70.0	23.4	0	6.6

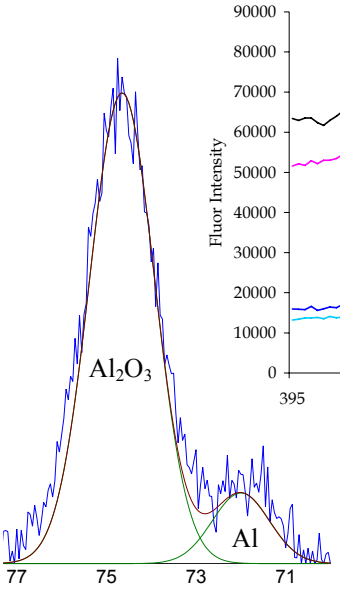

Figure 1. Al 2p high resolution ESCA peak of O_2/H_2O-plasma treated aluminum substrate.

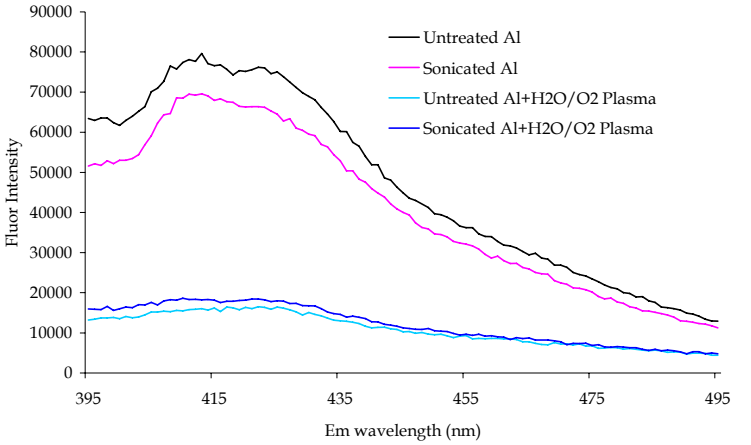

Figure 2. Fluorescence spectra of untreated, sonicated and water / oxygen plasma treated samples after labeling them with 9-antracene carboxylic acid.

It was shown that only plasma species and not sonication were responsible for the extensive removal of hydrocarbon-type, contamination structures from aluminum surfaces. However, the photoluminescence of dyes is strongly diminished by adsorbing them onto inorganic solid surfaces [14]. It is suggested that the etching effect of plasma and the removal of organic contaminants from the substrate surfaces contributed probably to the intense adsorption of dye molecules. The presence of 9-anthracene carboxylic acid attached on the plasma-modified surfaces is demonstrated by survey and HR ESCA data (Figure 3). A significantly increased relative carbon atomic concentration of the anthracene-containing samples in comparison to the oxygen/water plasma treated substrates and the presence of –COOH functionalities (291 eV) is indicative for the existence of anthracene structure. The 289.2 eV and 284.7 eV BE peaks are indicative for the existence of –COOH and –C=C- linkages.

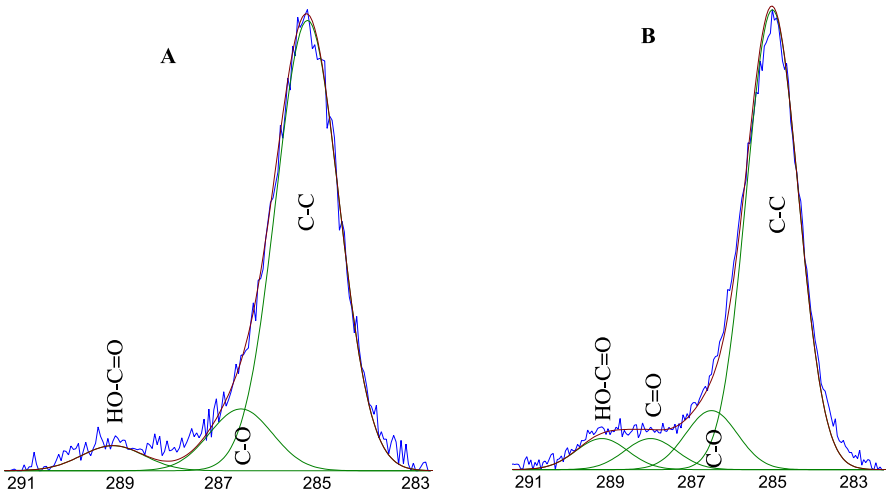

Figure 3. C 1s ESCA spectra of sonicated and oxygen/water vapor plasma-exposed aluminum (A) and plasma-treated aluminum coated with anthracene carboxylic acid and subsequently ultrasound washed (B).

A typical HR fitted C1s spectra of the H_2O/O_2-plasma treated, PAA-coated and heated sample that was ultra sound- assisted water washed is presented in Figure 4. ESCA diagram shows the existence of all three BE peaks [15] characteristic for the PAA structure (CH_2 and CH: 285; C*C=O: 285.6 eV; and CH-*CO-OH: 289.2 eV). The relative surface atomic composition of PAA-, PVP-, and PAA/PVP-coated substrates (Table 1) is also indicative for the presence of PAA, PVP, and PAA/PVP structures on the specifically modified substrate surfaces. Carbon contamination of oxygen/water plasma treated aluminum surfaces is due to the sample handling through atmospheric laboratory environment.

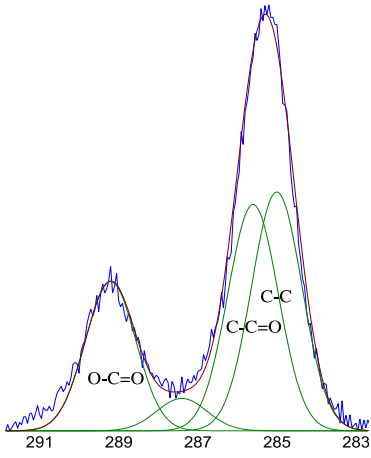

Figure 4. C 1s ESCA spectra of PAA-coated aluminum sample.

Figure 5 exhibits the color of plasma-treated and plasma-treated and PAA-coated and washed substrates that were subsequently exposed to o-toluidine [9]. The violet color is indicative of the presence of PAA on the aluminum surface. By the toluidine blue O staining method, an average of 143 µg PAA was estimated to be present on each PAA-coated substrate (28.22 µg/cm^2).

For comparison reasons the HR C1s and N1s ESCA spectra of PVP deposited (deep-coated and room temperature and vacuum oven-dried without future treatment) onto the aluminum surface has also been recorded (Figure 6). It can be observed that the three peaks resulting from curve-fitting (Table 2) match the BE values of the carbon atoms of the PVP structure (CH_2: 285 eV; CH aliphatic and aromatic: 285.5 eV, and C-N: 286 eV). The differences in the relative surface areas of the specific BE peaks

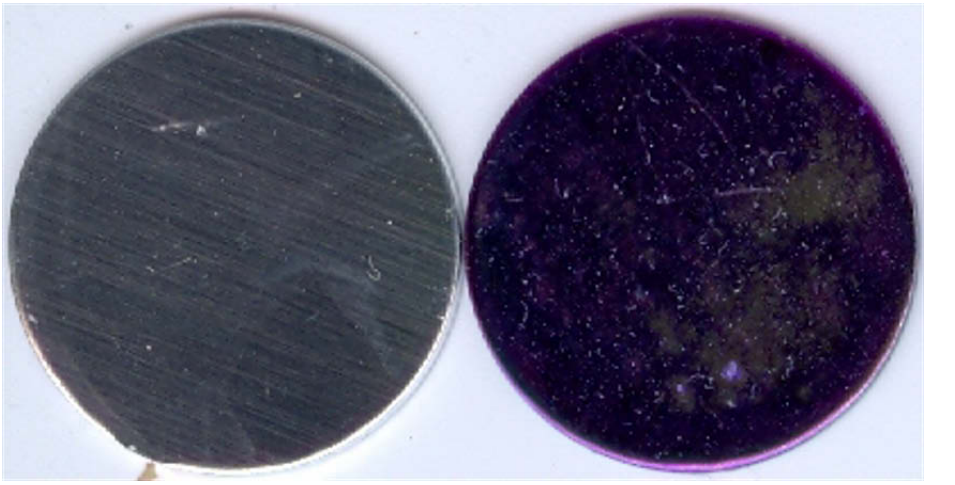

Figure 5. Unmodified, plasma-treated and plasma-treated and subsequently PAA-coated and washed substrates that were subsequently exposed to o-toluidine.

both in the PAA and PVP HR diagrams in comparison to the theoretical structures can be related to the presence of contaminants or to the selected curve-fitting process. The existence of a symmetrical N1s 399.4 eV BE peak in the HR, N1s PVP spectra substantiate the presence of PVP structure (Figure 6).

Table 2. Relative area of the peaks resulting from curve-fitting of C1s ESCA peak of PAA, PVP and PAA/PVP samples.

Sample	C-C	CH_2-CH-*C(Ar) and CH2- *CH-COOH	C=N	C=O	O-C=O
	285 eV	285.6 eV	286 eV	287.4 eV	289.2 eV
PAA	36.9	35.0	-	5.0	23.1
PVP	28.9	44.4	26.6	-	-
PAA+PVP	33.5	35.7	17.5	3.3	10.0

HR ESCA data resulting from plasma-treated aluminum substrates that were subsequently coated with PAA and PVP indicate the co-existence of the two macromolecular structures on the substrate surfaces (Figure 7). CH_2, CH, C-N and CO-OH BE peaks are all present in the spectra. The symmetrical N1s peak (Figure 7) is also representative for the presence of PVP structure. However, the BE value of the N1s peak is shifted to a higher value (399.9 eV). This suggests that in addition to the hydrogen bonding-based interaction between the PAA and PVP macromolecules ionic attraction might also be present.

It should be emphasized that the PVP was present on the aluminum substrate surfaces that were pre-coated with PAA, even after a thorough ultra sound assisted washing (10 min) of the samples. Survey ESCA data indicate that a 2.2 % nitrogen surface atomic concentration was present after ultrasound washing relative to the 3.5% nitrogen atomic concentration rinsed only with water in the absence of ultrasound.

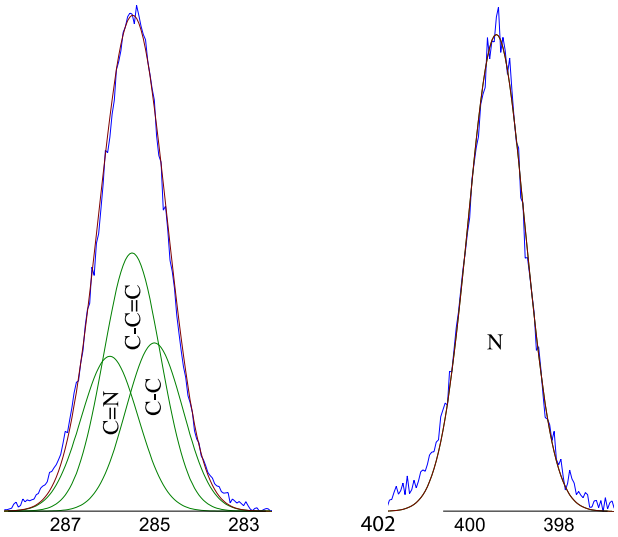

Figure 6. High resolution C1s and N1s ESCA spectra of PVP deposited on aluminum.

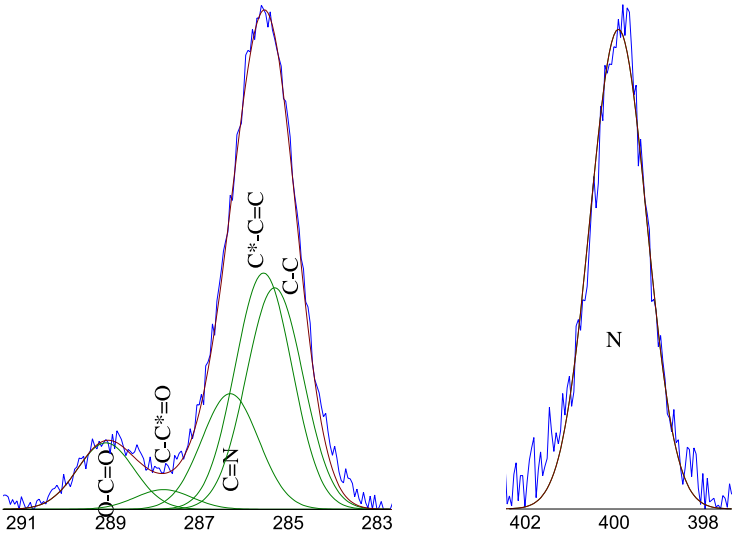

Figure 7. High resolution C1s and N1s ESCA spectra of PAA/PVP deposited on aluminum.

Contact angle evaluations (Table 3) of PAA-coated surfaces that were washed with distilled water and those washed with buffer after 24 hours clearly indicate the presence of higher contact angle values associated with the water-washed substrates. This allows us to assume that ionic strength can sensibly influence the orientation of the PAA macromolecules attached to the aluminum surfaces and that their effect might also influence the bactericidal behavior of the samples. Further investigations will be focused in this direction.

Table 3. Contact angle (degrees) evaluations of PAA-coated surfaces.

Untreated Al	PAA-Coated Al Immediately after H_2O washing	PAA-Coated Al 48 h after H_2O washing	PAA-Coated Al Immediately after buffer washing	PAA-Coated Al 48 h after buffer washing
85±5	15.6±6	44.2±2	0	26.5±4

SEM images of uncoated and PAA coated aluminum substrates are presented in Figure 8. It can be noted much higher roughness and complex topography at micro- and macro-scale (μm and higher) for the PAA coated sample in comparison to the uncoated substrate.

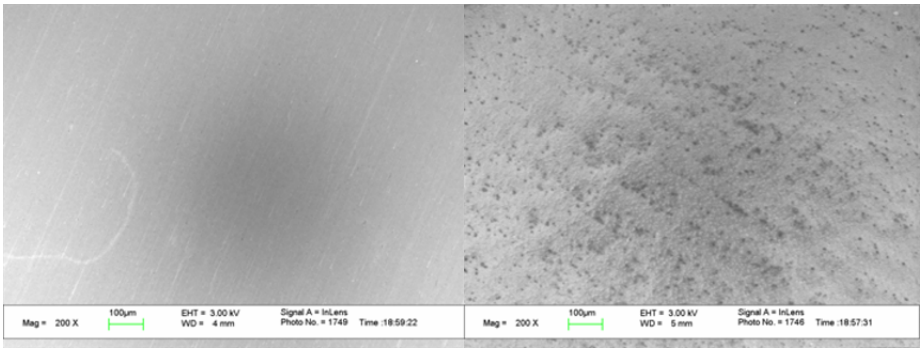

Figure 8. SEM images of uncoated (left) and PAA coated mirror-polished aluminum substrates; Magnification: ×200.

The antibacterial activity of PAA-coated substrates is presented in Tables 4 and 5. About 82 to 98% decrease in bacterial populations was observed after exposure to the surfaces for 24 hours.

Table 4. Bactericidal effect of PAA-coated aluminum substrates (n = 4) on *L. monocytogenes.*

	Log cfu/substrate	Log decrease	% decrease
Control	5.14	0	0
Plasma treated	5.24	0	0
Plasma and PAA treated	3.39	1.75	98.2

Table 5. Bactericidal effect of PAA-coated aluminum substrates (n = 2) on a mixture of *E. coli*, *P. aeruginosa* and *S. epidermidis.*

	Log cfu*/substrate	Log decrease*	% decrease*
Control	6.13/4.09	0	0
Plasma and PAA treated	4.74/3.35	1.39/0.74	96.0/81.9

*First number is for both *E. coli* and *P. aeruginosa*; second number is for *S. epidermidis*.

Conclusions

PAA molecules were attached to oxygen/water vapor-plasma modified aluminum surfaces. It was demonstrated that PVP can be subsequently attached to PAA coated surfaces with the generation of stable bi-layer structures. High resolution ESCA data collected from PAA/PVP-coated aluminum allow us to suggest that in addition to hydrogen bonding ionic forces might also play a role in the interaction of PAA and PVP layers. The bactericidal behavior of PAA coated aluminum was evaluated ant it was shown that PAA coated substrates have anti-bacterial characteristics. Experiments are under way for the plasma-enhanced coating of silicon surfaces with PAA/PVP layers. This plasma-enhanced approach opens up additional possibilities for the attachment of polycarboxylic macromolecular structures to various substrate surfaces (e.g. silicon) for biotech applications.

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