Plasma Polymer Film Structure and DNA Probe Immobilization

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ABSTRACT: The present work describes the fabrication, characterization, and optimization of NH_2-derivatized polymer coatings prepared by pulsed-plasma polymerization for applications as adhesion layers in DNA immobilization. Fourier transform infrared spectroscopy and surface plasmon resonance spectroscopy were used to study (i) polymer matrix properties and (ii) oligonucleotide/DNA binding. The successful DNA attachment on amine functionalized surfaces was found to depend on the macromolecular architecture of the plasma films and on the amine group densities. Pulsed and continuous wave plasma polymers deposited at similar equivalent power showed comparable immobilization properties, while low duty cycle plasma-polymerized films showed a higher sensitivity toward DNA binding than high duty cycle plasma-deposited films. The stabilities of the various films in buffer solution and their reactivities as they were affected by variations in pH have been investigated.

1. Introduction

The controlled attachment of biological molecules on plasma-polymerized thin organic films has received increased attention over the past years. While plasma polymerization processes enable the deposition of a tremendous variety of functional thin films[1−10] on virtually any substrate, the application of these deposits often suffers from insufficient adhesion to the substrate and from often unpredictable swelling behavior when subjected to aqueous solution. Detailed in situ studies of the adsorption of biological molecules on plasma polymer surfaces are further hindered by the lack of analytical tools suitable for measurement of ultrathin films in solution with nanometer resolution. In the past, we have described the use of impedance spectroscopy[11] and optical methods such as surface plasmon resonance spectroscopy (SPR)[12] and waveguide mode spectroscopy (WaMS)[13] to study the properties of plasma polymer films in solution. A number of methods are available to overcome the problem of adhesion to the substrate: (a) pretreatment of the substrate in an activating plasma (Ar or O_2), suitable for polymeric substrates, or (b) the use of an additional layer which binds covalently to the substrate surface on one side and to the plasma polymer on the other side. Examples of this using self-assembled short chain thiols on gold substrates have previously been described.[11]

It has previously been shown that pulsed-plasma polymerization gives a remarkable control over the chemical structure of the deposited films, in particular for deposits at low duty cycle (DC = $t_{on}/t_{on}+ t_{off}$).[13−18] This is because of the combined effects of the DC and the applied power, which allows equivalent powers ($P_{eq} = P_{peak} \times$ DC) of $\ll 5$ W to be used. By careful control of the process parameters, it is principally possible for the plasma t_{on} phase to be used as an activation process and the subsequent t_{off} phase to be used for thin film deposition by a radical polymerization process.

Electrochemical and optical measurements of the films in solution have previously shown that plasma polymer films deposited at low DC swell considerably and exhibit hydrogellike character when submersed in aqueous solution.[11,19] This property decreases with increasing DC and was not observed for high power plasma deposits. The unique properties of functional plasma polymer films makes them particularly attractive for use as supports for biological material and efforts are underway to investigate the applicability of these materials for biomedical devices and sensors. Work by a number of researchers has shown plasma polymer films to be highly suitable as antifouling surfaces and considerable control over cell adhesion has been demonstrated.[19−27] The present paper investigates the adsorption of single-stranded DNA on pulsed-plasma-polymerized allylamine films under different aqueous environments and with respect to different deposition conditions. In situ SPR measurements were used to study the behavior of pulsed-plasma-polymerized allylamine films in solution and to correlate DNA adsorption to the chemical nature of the plasma films. The results are correlated to chemical structure analysis using Fourier transform infrared spectroscopy.

2. Experimental Section

All chemicals were purchased from Sigma-Aldrich, Deisenhofen, Germany. The DNA probe samples used were purchased from MWG BIOTEC AG, Ebersberg, Germany

Substrates. The substrates used throughout the work were LaSFN9 optical glass slides coated with approximately 50 nm of gold. To ensure optimal adhesion of the plasma polymer to the gold surface a monolayer of octadecanethiol was allowed to self-assemble on the gold surface.[11] These substrates were used in subsequent plasma polymerization reactions immediately after preparation.

Plasma Polymerization. The plasma polymers were prepared in a cylindrical (300 mm long, o.d. 100 mm) 13.56 MHz rf plasma reactor either under continuous wave (cw) or pulsed-plasma conditions. The samples were placed halfway between the electrodes, which consisted of two concentric rings wrapped around the outside of the chamber and separated by 12 cm.[13] The input power (P_{peak}) used in the continuous wave plasma deposition experiments were 5 and 100 W. During the pulsed-

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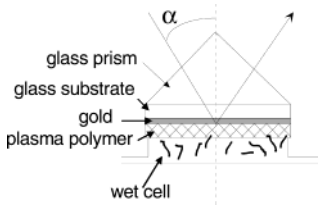

Figure 1. Schematic of the SPR setup.

plasma-polymerization (ppp) experiments, duty cycles (DC = $t_{on}/t_{on}+ t_{off}$) of 10/50 and 10/200 were used. The equivalent power ($P_{eq} = P_{peak} \times$ DC) during the pulsed experiments were thus 20 and 2.5 W respectively. The plasma process pressure used was 0.13 mbar and 0.06 mbar for some 10/50 ppp−allylamine films. Deposition times ranged between 30 s and 15 min.

Film Characterization. The film chemical structure was analyzed by Fourier transform infrared spectroscopy (FTIR) using a Nicolet 850 spectrometer**.** Relative areas of the absorption bands around 3300 cm^{-1} (for $-NH_2$ groups) and at 2900 cm^{-1} (CH_x groups) were used to estimate and maximize the approximate amine functional group densities. Film thicknesses were measured by surface plasmon resonance spectroscopy (SPR). The adsorption of DNA probes was monitored using SPR equipped with a wet cell made of Teflon sealed with a viton O-ring. Measurements were made using a continuous flow of buffer or buffer/DNA mixtures over the polymer surface.

Surface plasmon resonance spectroscopy and its applicability in surface and interface analysis has been described elsewhere.[12] It is based on the electromagentic mode that propagates along a metal surface and that is associated with an evanescent wave with a decay length normal to the surface of 150−200 nm. The excitation of the surface plasmon is very sensitive toward changes in the refractive index of the medium sensed by the evanescent wave in close proximity to the gold surface. Monochromatic, linearly (p-) polarized light is directed through a quartz prism such that under total internal reflection the surface plasmon is excited at the gold/dielectric interface at a certain angle of incidence resulting in a sharp minimum in the intensity of the reflected light, Figure 1. If the refractive index of the medium just outside the gold surface changes, e.g., due to swelling or DNA adsorption, a proportional change in the angle ($\Delta\alpha$) can be observed. Attachment of a suitable Teflon wet cell, sealed with a Viton O-ring and equipped with a buffer flow system, allows the SPR technique to give for real-time measurements of the DNA adsorption to the surface. All samples were mounted into the spectrometer and the film equilibration in PBS solution under a dynamic flow was monitored. Once the film had stabilized, the previously prepared DNA solution was passed through the cell. Time-dependent measurements of DNA uptake were made by measuring the changes in the reflected light at a fixed angle of incidence (α). The measured % reflected light is proportional to the optical thickness (*nd*) of the surface layer and can be utilized to make assumptions about the equivalent thickness of the adlayer binding at the interface.[12] Once no more changes were observed in the reflected light, DNA binding was assumed to be complete, and the wet cell was rinsed with four cell volumes of PBS buffer solution to remove excess DNA molecules.

DNA Samples. The DNA probe samples were stored at −4 °C until use. The oligomers used were 30mer and 25mers, though some selected experiments were carried out using a 60mer.

The DNA solutions were made up using phosphate buffer solutions consisting of (A) 0.2 mol/L NaH_2PO_4 (27 g in 1 L of H_2O) and (B) 0.2 mol/L Na_2HPO_4 (53.65 g $Na_2HPO_4 \cdot 7H_2O$, or 71.7 g $Na_2HPO_4 \cdot 12H_2O$ in 1 L of H_2O. These were mixed according to the following recipes depending on the pH required: (i) dilute A to give 0.1 mol/L buffer at pH 4.5; (ii) 93.5 mL of A and 6.5 mL of B plus 100 mL of H_2O giving 200 mL of 0.1 mol/L buffer of pH 5.7; (iii) 39.0 mL of A and 61.0 mL of B plus 100 mL of H_2O giving 200 mL of 0.1 mol/L buffer of pH 7; (iv) dilute B to give 0.1 mol/L buffer at pH 9.

Table 1. Plasma Polymerization Conditions, FTIR, and Contact Angle Data for Different cw and Pulsed Plasma Polymerized Allylamine Films

duty cycle	P_{peak}/W	P_{eq}/W	% rel area from 3100 to 3500 cm^{-1} (NH_2 band) ± 1%	θ_A/° (±3°)
cw	100	100	14	79
10/50	100	20	16	60
cw	5	5	25	29
10/50	5	1	24	30
10/200	50	2.5	26	33

Polylysine Film Preparation. Poly-L-lysine (MW 3400) was self-assembled onto the gold-coated glass substrates from a solution of 5 mg/L in PBS. The samples were rinsed in excess milli-Q water, and then mount into the SPR setup for further measurements. This typically lead to a PL- film thickness of approximately 2 nm.

3. Results and Discussion

Plasma Polymer Film Structure and Properties. The FTIR spectra of plasma-polymerized allylamine using different plasma conditions, as shown in Figure 2, allow some assumptions to be made about the chemical nature of these films. The improved retention of the monomer structure with decreasing DC can be seen by the increasing intensity of the band around 3300 cm^{-1} ($-NH_2$ groups) accompanied by decreasing intensity of the bands around 2900 cm^{-1} (hydrocarbon) and 2100 cm^{-1} (C═N/C═O bonds associated with monomer degradation), Figure 2a. The low relative intensity of the CH_x band (∼2900 cm^{-1}) suggests the low DC films to have a low cross-link density. With increasing duty cycle the bands at 2900 and 2100 cm^{-1} increased in relative intensity, while that of the $-NH_2$ (3300 cm^{-1}) component decreased, indicating considerable degradation of the monomer and the formation of films with increasing cross-link density.

The FTIR spectra of plasma polymer films deposited under continuous wave conditions, Figure 2b shows that at the comparatively high input power of 100 W the film chemical structure appears to be similar to that of the high DC (10/50, P_{eq} 20 W) plasma polymer film. Similarly, under low power cw conditions (5 W) the FTIR spectra show a film chemical structure that is comparable to that of the low DC (P_{eq} 2.5 W).

A semiquantitative calculation of the relative peak areas within the normalized FTIR spectra, Table 1, showed the amino band (3300 cm^{-1}) observed for the low DC films (low P_{eq}) to contribute approximately 25% of the total overall spectrum, while it represented only approximately 15% of the overall spectrum for the high duty cycle (high P_{eq}) films. These observations were consistent with the contact angles observed: high DC films containing a low $-NH_2$ functional group density showed an advancing angle (θ_A) of >60°, while the low DC films with a substantially higher NH_2-functional group density were more hydrophilic with θ_A approximately 30°.

No significant differences could be observed between the cw 5 W and the low P_{eq} plasma polymer films by FTIR or contact angle measurements.

Plasma polymers have previously been reported to contain low molecular weight material which is not covalently bonded to the network or to the substrate and which can be removed by dissolution or solvent extraction[13,28] leading to an observed decrease in film thick-

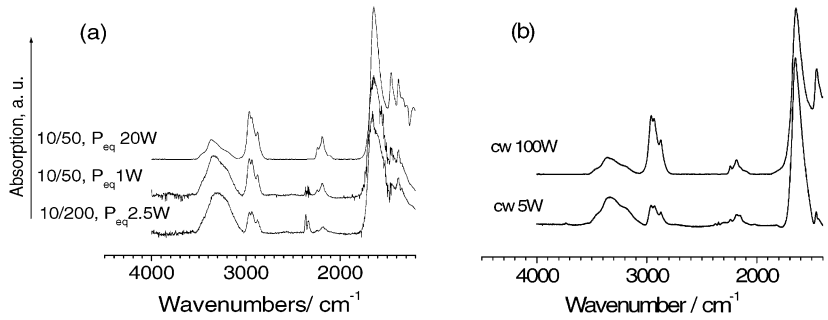

Figure 2. Normalized FTIR spectra of plasma-polymerized allylamine under (a) pulsed and (b) cw plasma conditions.

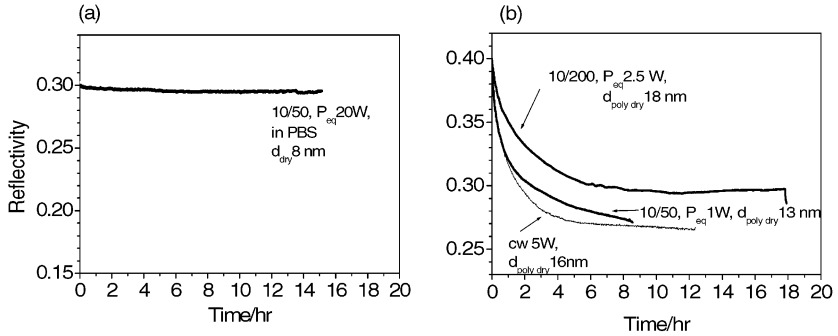

Figure 3. SPR data on the swelling behavior in PBS solution of (a) a high DC/high P_{eq} plasma polymer film and (b) different low DC/low P_{eq} plasma polymer films.

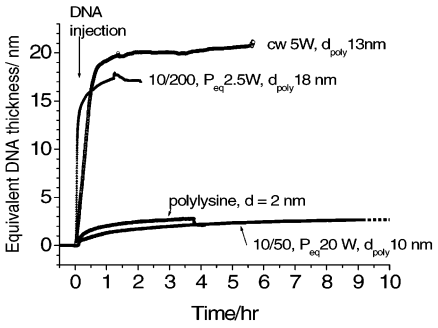

Figure 4. SPR kinetic measurements of DNA binding on a low DC (10/200), a 5 W cw and a high DC (10/50) plasma-polymerized allylamine compared to that on a polylysine film ($d \approx 2$ nm): C_{DNA} 100nM, PBS buffer at pH 7.4.

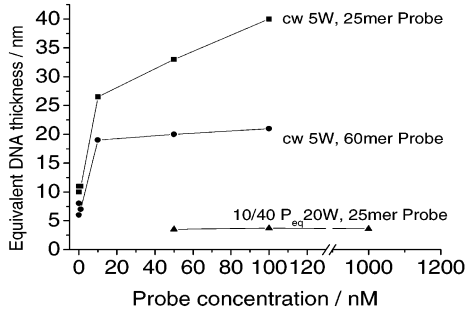

Figure 5. Dependence of the DNA equivalent thickness on the DNA probe concentrations on cw 5 W and high DC plasma films, $d_{polymer}$ 40 ± 3 nm, pH 7.4, shown for 25mer and 60mer DNA probe.

ness. While this appears to be generally true for plasma films deposited at low P_{eq}, surface plasmon resonance measurements in this work showed no significant changes in the optical thickness of high P_{eq} plasma films when submersed in aqueous buffer solution over a time period of up to 14 h, Figure 3a. The stability observed may arise from high molecular weight polymer chains and/or high cross-link density and thus a low freedom

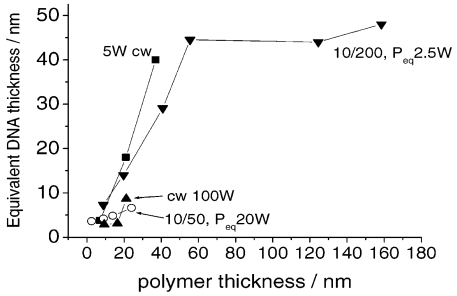

Figure 6. Dependence of the DNA equivalent thickness on the plasma polymer thickness for DNA adsorption on high and low duty cycle plasma films: C_{DNA} 100 nM; pH 7.4.

of movement within the network. Any unbonded material which may be present probably remains trapped within this relatively rigid network. This appears to be in agreement with the FTIR spectra above, which showed substantial monomer degradation and a high cross-link density.

The low DC and cw 5 W plasma polymer films in the present work, showed a 30−40% decrease in the optical thickness with exposure to aqueous buffer, Figure 3b. This appears to agree with a polymer network of low cross-link density (see the FTIR data above), consisting of relatively shorter chain molecules and a comparatively high freedom of movement within the network. The differences in the change in reflectivity (or Δnd) observed between the three low P_{eq} films may suggest subtle differences in the chemical nature of the films, however, the significance of these differences is at present difficult to assess without absolute measurements of refractive index and film thickness. The "swelling" characteristics of the plasma polymer films were taken into account in all DNA experiments and all low DC films were equilibrated for at least 6 h before further use.

DNA Immobilization. After equilibration of the polymer films, the kinetics of DNA probe attachment

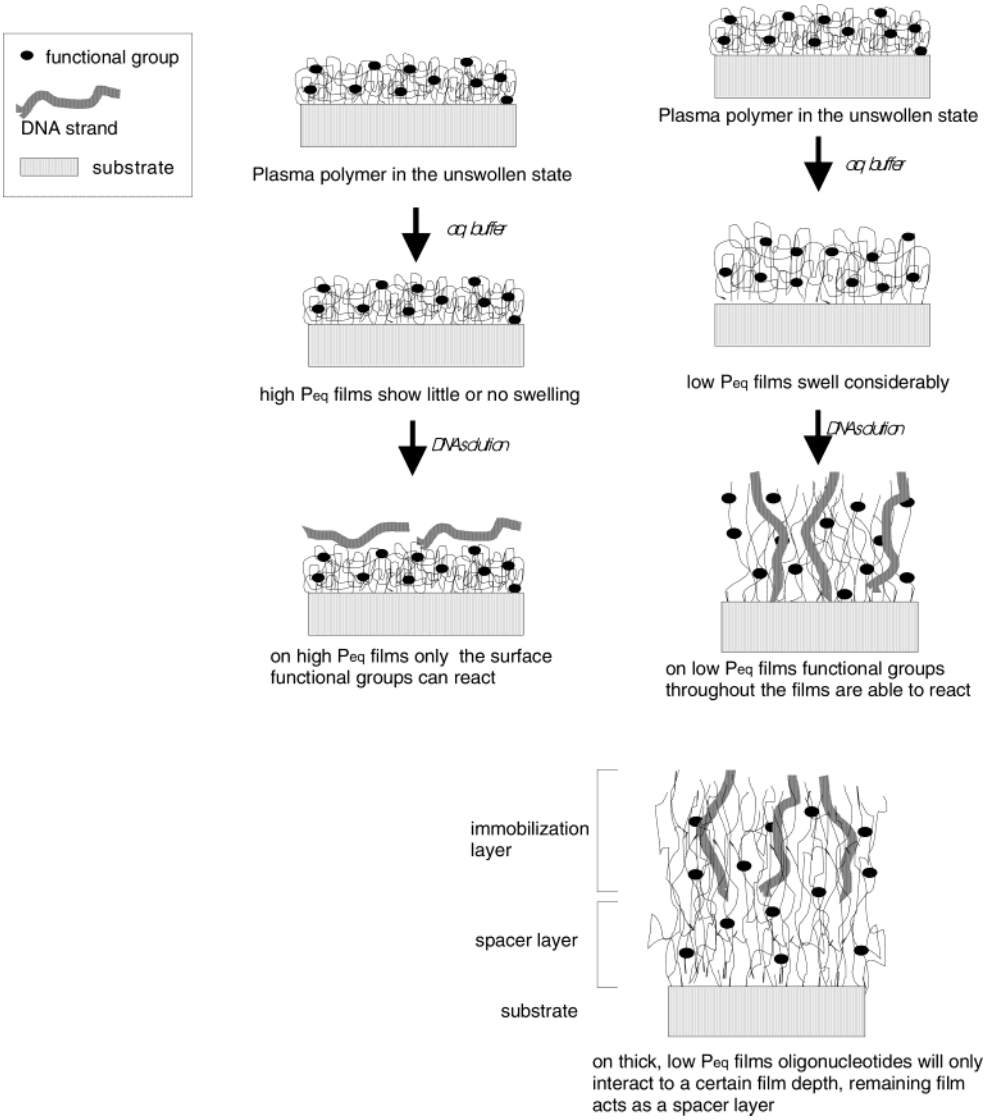

Figure 7. Schematic representation of plasma polymer swelling and DNA binding to low and high DC plasma polymer films.

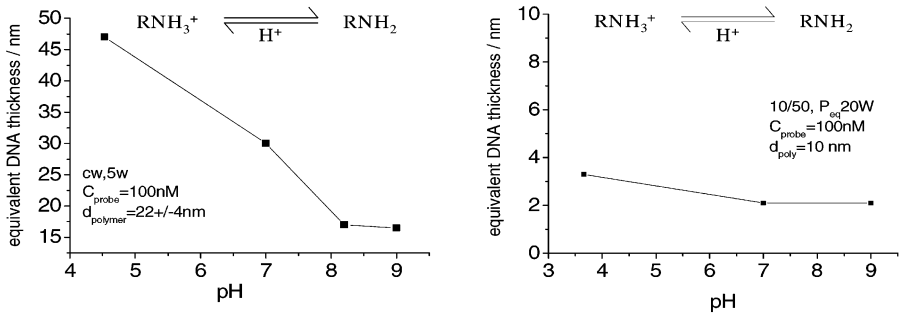

Figure 8. Effect of pH on the DNA adsorption on (a) 5 W cw films and (b) on high DC/high P_{eq} plasma polymer films.

were studied using a probe concentration of 100 nM. To calculate DNA thicknesses an average refractive index of $n = 1.485$[13] was assumed for the pp-allylamine films and $n = 1.375$ for the DNA adlayer.[29] DNA adsorption was found to be substantially more efficient for low DC films than for high DC (high P_{eq}) films as seen in the kinetic scan in Figure 4. The data were compared to the immobilization of DNA on poly-L-lysine keeping all other conditions constant. As is evident from the data in Figure 4, the high DC plasma polymer appears to exhibit similar binding properties as the polylysine film, both showing a optical thickness increase of approximately 3 nm. The equivalent thickness of the DNA layer measured for the low DC film, however, showed a 6-fold increase and for an 18 nm thick plasma polymer film a typical DNA thickness of 17 nm was observed. A comparable 5 W cw plasma polymer film showed very similar DNA adsorption with a total of approximately 20 nm DNA adsorbed.

Decreasing the probe concentration was found to lead to a decrease in the optical thickness of the bound oligonucleotides, as shown for the 5 W cw film in Figure 5. However, even for probe concentrations of 0.01 nM the probe thicknesses measured were still larger than

any values measured for the high DC films or for polylysine. It is interesting to note that increasing the DNA strand length from a 25mer to a 60mer decreased the DNA thickness measured by approximately a factor 2. This may suggest a relationship between DNA strand length and its accessibility into the polymer network. Variations in the probe concentration (1000−50 nM) had no significant effect on oligonucleotide adsorption on the high DC plasma films and the effective oligonucleotide thickness always remained approximately 3−4 nm.

The DNA adsorption was found to be dependent not only on the DC and the probe concentration but also on the polymer film thickness as shown in Figure 6. For polymers of effective thickness $d < 10$ nm (after swelling) the DNA thickness measured on the low and high DC plasma polymers were similar and within a few nanometers. However, with increasing polymer thickness, the low DC films indicated a much higher affinity for DNA probe attachment. A similar trend could be observed for the 5 W cw films studied, which even showed some enhanced binding over the low DC films. The adsorption of DNA could be studied by the SPR technique only up to a polymer thickness of approximately 50 nm due to the limited sampling depth of the evanescent wave. For polymer thicknesses >50 nm, the DNA adsorption was monitored using optical waveguide mode spectroscopy.[30,31] The total amount of DNA able to adsorb on a low DC plasma film (10/200) at a probe concentration of 100 nM reached a maximum for polymer films thicknesses of approximately 50 nm, showing an effective thickness increase due to oligunucleotide binding of approximately 45 nm.

The degree of immobilization is believed to be related to the cross-link density and the swelling characteristics of the plasma polymer films. Since the low P_{eq} polymers are less cross-linked and the chains have a greater freedom of mobility, the network can expand in solution making the functional groups within the bulk of the swollen polymer network more accessible to the DNA molecules. The oligonucleotides are thought to penetrate into the film as shown schematically in Figure 7. The data in Figure 6 suggest that for a specific probe concentration there appears to be an optimum plasma polymer thickness. Further increases in the plasma polymer film thickness will lead to no further improvement in oligonucleotide binding, but may provide an additional, relatively mobile and fluid "spacer layer" (see Figure 7). This "spacer layer" is not accessible to the oligonucleotides, but may act as a buffer reservoir for the system and may reduce surface effects or quenching of fluorescent labels when in close proximity to the gold substrate.

In contrast, the high DC films which are typically highly cross-linked show very little dependence on the polymer thickness. This can again be understood when considering the swelling behavior of these films (Figure 3), which suggested a rigid structure and a low freedom of movement of the chains within the polymer matrix. The DNA molecules are thus unable to bind to functional groups within the polymer network and are predominantly binding to sites on the uppermost surface of the film. Once a monolayer of DNA has adsorbed at the interface, the highly negatively charged surface adlayer prevents the binding of further DNA molecules.

When plasma-polymerized allylamine is subjected to buffers at different pH protonation of the amine group at low pH will lead to a highly positively charged film.

$$R-NH_2 \leftrightarrow R-NH_3^+$$

This increased positive charge was found to lead to enhanced binding of the negatively charged DNA to the polymer film, Figure 8. Keeping all other conditions constant and only changing the buffer pH, the thickness of DNA probe measured on low DC plasma polymers increased significantly. In contrast, the pH effect was significantly less pronounced on the highly cross-linked, high DC films even though the general trend was the same. This appears to be in agreement with the estimated relative functional group densities [32] and the accessibility of the groups within the two types of films as discussed above.

4. Conclusions

Plasma-polymerized, amine-functionalized films appear to exhibit properties which render them suitable for DNA adsorption. DNA probe immobilization was found to be optimal for low duty cycle pulsed or low power continuous wave plasma-deposited films with a relatively high density of $-NH_2$ groups, low contact angles, and a low cross-link density. In solution, the polyelectrolytic and hydrogel characteristics of these films seem to enhance DNA probe adsorption. The data appear to suggest that the oligonucleotides are able to penetrate into the polymer network and are able to interact with reactive sites to an effective film thickness of up to approximately 40−50 nm. This does not seem to be possible with the highly cross-linked, high P_{eq} films. Low pH values seem to favor DNA probe attachment.

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References and Notes

(1) Alexander, M. R.; Duc, T. M. *J. Mater. Chem.* **1998**, *8*, 937−943.
(2) Beck, A. J.; Short, R. D.; Jones, F. R.; France, R. M.; Kettle, A. P.; Daw, R.; O'Toole, L.; Dawson, R.; MacNeil, S. *Mater. World* **1998**, *6*, 70−72.
(3) Calderon, J. G.; Timmons, R. B. *Abstr. Pap. Am. Chem. Soc.* **1997**, *213*, 534-POLY.
(4) Calderon, J. G.; Harsch, A.; Gross, G. W.; Timmons, R. B. *J. Biomed. Mater. Res.* **1998**, *42*, 597−603.
(5) Calderon, J. G.; Timmons, R. B. *Macromolecules* **1998**, *31*, 3216−3224.
(6) Candan, S.; Beck, A. J.; O'Toole, L.; Short, R. D. *J. Vac. Sci. Technol. a−Vac. Surf. Films* **1998**, *16*, 1702−1709.
(7) Chen, X. L.; Rajeshwar, K.; Timmons, R. B.; Chen, J. J.; Chyan, O. M. R. *Chem. Mater.* **1996**, *8*, 1067−1077.
(8) Cho, D. L.; Yasuda, H. *Abstr. Pap. Am. Chem. Soc.* **1987**, *193*, 99-PMSE.
(9) Coulson, S. R.; Woodward, I.; Badyal, J. P. S.; Brewer, S. A.; Willis, C. *J. Phys. Chem. B* **2000**, *104*, 8836−8840.
(10) Coulson, S. R.; Woodward, I. S.; Badyal, J. P. S.; Brewer, S. A.; Willis, C. *Chem. Mater.* **2000**, *12*, 2031−2038.
(11) Jenkins, A. T. A.; Hu, J.; Wang, Y. Z.; Schiller, S.; Foerch, R.; Knoll, W. *Langmuir* **2000**, *16*, 6381−6384.
(12) Knoll, W. *Annu. Rev. Phys. Chem.* **1998**, *49*, 569−638.
(13) van Os, M. T.; Menges, B.; Foerch, R.; Vancso, G. J.; Knoll, W. *Chem. Mater.* **1999**, *11*, 3252−3257.
(14) Ryan, M. E.; Badyal, J. P. S. *Macromolecules* **1995**, *28*, 1377−1382.
(15) Ryan, M. E.; Hynes, A. M.; Badyal, J. P. S. *Chem. Mater.* **1996**, *8*, 37−42.
(16) Savage, C. R.; Timmons, R. B.; Lin, J. W. *Chem. Mater.* **1991**, *3*, 575−577.
(17) Panchalingam, V.; Chen, X.; Savage, C. R.; Timmons, R. B. *Abstr. Pap. Am. Chem. Soc.* **1993**, *205*, 228-POLY.

(18) Panchalingam, V.; Poon, B.; Huo, H. H.; Savage, C. R.; Timmons, R. B.; Eberhart, R. C. *J. Biomater. Sci.—Polym. Ed.* **1993**, *5*, 131−145.
(19) Zhang, Z.; Menges, B.; Timmons, R.; Knoll, W.; Förch, R. *Langmuir* **2003**, *19*, 4765−4770.
(20) Favia, P.; Sardella, E.; Gristina, R.; Milella, A.; d'Agostino, R. *J. Photopolym. Sci. Technol.* **2002**, *15*, 341−350.
(21) Favia, P.; d'Agostino, R. *Vide: Sci. Tech. Appl.* **2002**, *57*, 40−48.
(22) Favia, P.; d'Agostino, R. *Surf. Coatings Technol.* **1998**, *98*, 1102−1106.
(23) Yeh, Y. S.; Iriyama, Y.; Matsuzawa, Y.; Hanson, S. R.; Yasuda, H. *J. Biomed. Mater. Res.* **1988**, *22*, 795−818.
(24) Yeh, Y. S.; Iriyama, Y.; Yasuda, H.; Matsuzawa, Y.; Hanson, S. R. *Abstr. Pap. Am. Chem. Soc.* **1987**, *193*, 162-PMSE.
(25) Beyer, D.; Knoll, W.; Ringsdorf, H.; Wang, J. H.; Timmons, R. B.; Sluka, P. *J. Biomed. Mater. Res.* **1997**, *36*, 181−189.
(26) Daw, R.; Brook, I. M.; Devlin, A. J.; Short, R. D.; Cooper, E.; Leggett, G. J. *J. Mater. Chem.* **1998**, *8*, 2583−2584.
(27) Carlisle, E. S.; Mariappan, M. R.; Nelson, K. D.; Thomes, B. E.; Timmons, R. B.; Constantinescu, A.; Eberhart, R. C.; Bankey, P. E. *Tissue Eng.* **2000**, *6*, 45−52.
(28) van Os, M. T.; B. M.; Foerch, R.; Timmons, R. B.; Vancso, G. J.; Knoll, W. *Mater. Res. Soc. Symp. Proc.* **1999**, *544*, 45−50.
(29) Zizlsperger, M. Ph.D. Thesis, Johannes-Gutenberg-Universität Mainz, Germany, 1998.
(30) Knoll, W. In *Handbook of optical properties*; Wissmann, R. E. H. P., Ed.; CRC Press: London, New York, and Tokyo, 1997; Vol. II, pp 373−399.
(31) Aust, E. F.; Sawodny, I. S.; Knoll, M. W. *Trends Polym. Sci.* **1994**, *2*, 313−323.
(32) Schönherr, H.; van Os, M. T.; Hruska, Z.; Kurdi, J.; Förch, R.; Arefi-Khonsari, F.; Knoll, W.; Vansco, G. J. *Chem. Commun.* **2000**, 1303−1304.

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