

Stability of an Ultrathin Plasma Polymerized Film in Aqueous Solution: In Situ Detection by Surface Plasmon Resonance

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Recently, surface plasmon resonance (SPR) has been successfully implemented to characterize the film stability in real time: a radio frequency (RF) plasma polymerized film was monitored by swelling or extracting behaviors in aqueous solution. The high-resolution SPR revealed that film stability strongly depended on the monomers, plasma mode, and substrate locations as well as plasma polymerization conditions: incident power and working pressure. By Fourier transform infrared (FTIR), the possible reasons of film stabilization affected by plasma conditions and sample locations are explained. It is recommended that as an adhesive layer for future applications the plasma polymerized polymer shall be prepared in low working pressure pulsed plasma.

Introduction

Today many methods are developing to diagnose the genetic lesions: thousands of different diseases are known to be caused by genetic lesions such as single point mutation in the human genome,^{1,2} whereas the conventional gel based sequencing methods are relatively expensive in terms of equipment and labor costs.^{3,4} With the application of laser technology novel genetic lesion detection formats on substrate surface were designed and promised to fulfill the requirement for an optimal biosensor. In these sensors surface plasmon resonance (SPR) is a typical successful as it is simple, rapid, label-free, and low-cost. SPR then is widely utilized in DNA surface hybridization^{5,6} and protein adsorption,⁷ and as antibodies and antigens.⁸

Recently we expected to utilize the SPR technology to monitor ultrathin film stability in aqueous solution. In our previous works,^{9,10} the DNA surface hybridization reaction research, the several nanometer plasma polymerization polyallylamine (PPAA) layer was proposed by replacing the universal binding matrix of streptavidin to the self-assembled biotinylated monolayer as an adhesive layer by electrostatic force based on DNA negatively charged backbone and protonated PPAA. It was noticed that the stabilization of the PPAA layer greatly dominates the SPR usability in DNA surface hybridization detection not only due to the nanometer scale DNA strands in the hybridization reaction but also the SPR sensitivity. The nanometer scale oligomers such as the 15-mer DNA probe that is generally used in DNA hybridization assay is only about 3.5 nm on the surface. This means a slight variation from the polymer swelling or extracting will mask the result from the DNA hybridization reaction and distort later biosensor assay data. The precise monitoring of the film stability in the aqueous solution therefore is another tough problem.

With its high resolution, in this paper the SPR is performed by monitoring the film stability, investigating the influences of plasma polymerization conditions on the nanometer scale film structure. The kinetic spectroscopy of the polymer swelling or extracting behavior in real time is followed by fixing the detector at a minimum external incidence angle and determining the reflectivity as a function of the time.¹¹ The possible deposition mechanism and comparison of the film composition between

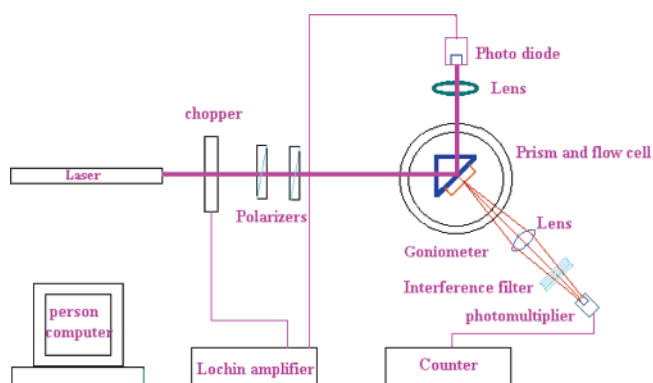


Figure 1. Scheme of the surface plasmon resonance detection unit that allows for the simultaneous recording of angular and kinetic scans at the base of the metal-coating coupling prism in this Kretschmann configuration.

films polymerized in continuous mode and pulsed plasma are also presented in the paper.

Experimental Section

The plasma reactor and associated electronics used in this work have been described in detail in ref 12. The 13.56 MHz capacitively coupled RF plasma discharge apparatus is home-built, consisting of a 300 mm length and 100 mm diameter Pyrex tube reactor and two outside electrodes separated ca. 12 cm. A substrate was located at the midpoint of the electrodes or downstream (ca. 40 cm from the grounded electrode) corresponding to the polymer polymerized in the middle or downstream. The continuous wave (CW) and pulsed plasma were chosen to study the influence of the plasma mode. In pulsed plasma the plasma-on time t_{on} varied from 1 to 10 ms while plasma-off times t_{off} were from 10 ms and 20 ms to 200 ms. The influence of working pressure was performed in 0.06 mbar and 0.12 mbar, referred to as the low and high working pressure, respectively. The kinetic swelling or extracting behavior of 3 to 7 nm thickness polymerized film monitored by SPR was implemented in a 1 mL flow cell in the static condition, i.e., the PBS solution was injected in the flow cell with no pumping

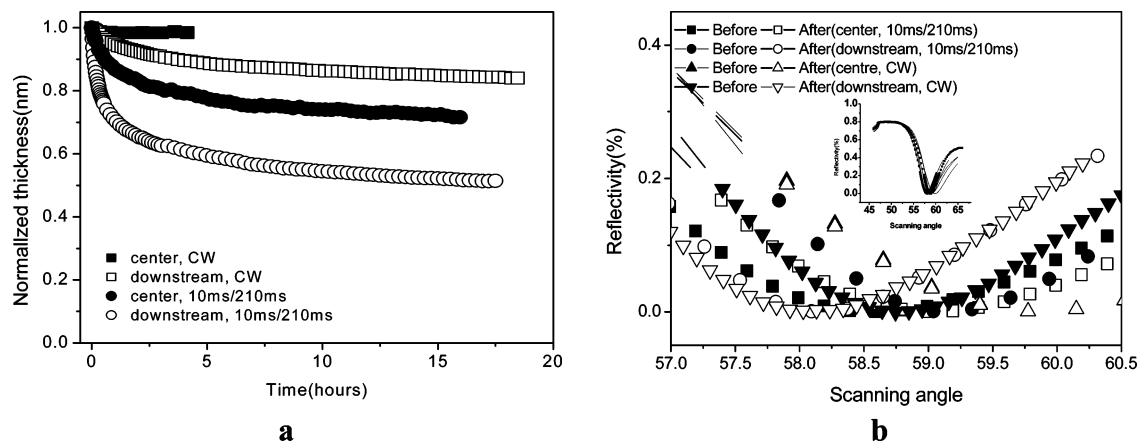


Figure 2. The reflectivity change as function of (a) kinetic mode for the different plasma deposited films on gold surface in aqueous solution and (b) scanning angle (the insert figure is a whole view).

circulation. At the equilibrium of swelling or extracting, ca. 200 times the flow cell volume of PBS solution was injected to rinse the sample and wash away the weakly binding molecules from the surface. The process was ended with a final SPR scanning if no visual variation appeared in the kinetic curve after rinsing.

The architecture of the substrate for SPR measurement is as follows:¹³ a film of ca. 47 nm Au was vacuum evaporated on the high refractive index glass LaSFN9 as substrate, then immersed into 10 mM octadecanethiol dichloromethane solution, forming a self-assembled monolayer on the gold surface to improve the adhesive between gold and the polymer, then it was put in the reactor and deposited functional film in the plasma. The FTIR measurement was carried on a Nicolet 850 instrument. For such measurement the polymer film was deposited on a glass—Cr—Au surface for reflective analysis. After the first FTIR measurement, the sample was immersed in PBS solution for 24 h, taken out and spun at 500 rps for 1 min, then heated at 50 °C in a vacuum overnight; the second FTIR measurement was performed to molecularly criticize the polymer stability in PBS solution. For atomic force microscopy (AFM) analysis, which also detected the surface stability from morphology by surface flatness and homogeneity, the polymer was deposited on gold-plated glass.

All chemicals used in this work are from Sigma-Aldrich without further purification.

Results

Figure 2 shows the relationship of the film stability with substrate locations. It found the film stability strongly depended on the film location. When the substrate was placed in the center of electrodes with monomer allylamine, 200 W peak power, CW plasma, and 0.12 mbar working pressure, the film appeared extremely stable in the aqueous solution. The kinetic curve decayed negligently in the whole period as Figure 2a shows. The scanning spectra in Figure 2b verifies no shifting of the dip angle before and after immersing in PBS solution for 4 h, i.e., no swelling or extracting reaction in the polymer. In contrast, the sample polymerized downstream with the same plasma conditions showed a remarkable dissociation reaction: the dip angle shifted 0.12° corresponding to a 15% decrease of the polymer thickness after 4 h.

Such behavior was much pronounced in the pulsed plasma polymerized polyallylamine film. Figure 2a reveals the film dissociated very quickly in PBS solution when substrates located in the center with 200 W peak power, 0.12 mbar pressure but 10 ms (t_{on})/210 ms ($t_{on} + t_{off}$) duty cycle pulsed plasma. The

TABLE 1: Derivative of the Relative Intensity of IR Absorption of $I_{NH_2(3360)}/I_{CH_3(2960)}$ and $C\equiv N/CH_3$ from Figure 3

deposition conditions	I_{NH_2}/I_{CH_3}	$I_{C\equiv N}/I_{CH_3}$		
		C≡N at 2153	C≡N at 2185	C≡N at 2240
1 ms/50 ms	1.210	0.264		0.01
10 ms/210	0.932	0.339	0.338	0.024
10 ms/50 ms	0.397		0.171	0.099
10 ms/30 ms	0.406		0.320	0.163
10 ms/20 ms	0.400		0.360	0.1784
CW	0.275		0.478	0.247

film thickness was reduced about 25% after immersing for 4 h, compared with 10% in CW plasma. When the film was polymerized downstream, moreover, the magnitude rose to 45% and the dip angle shifted 0.24° to the left.

From Figure 2 we now determine the plasma mode is very important in polymerizing the stable film. Due to the high densities and a long interaction period of electrons and ions bombarding and then cross-linking the films polymerized in CW plasma were significantly stable: either the substrate located in the center or downstream compared with the pulsed plasma mode—the films were always dissociation in the solution.

Besides the plasma mode playing an important role in the film stability, even in the pulsed plasma, furthermore, the duty cycles also dominated the polymer stability. In Figure 3a it can be seen that a higher duty cycle leads to a more stable polymer in pulsed plasma polymerization. For instance in the 1 ms/50 ms duty cycle and 200 W peak power the polymer dissociated rapidly after being immersed in PBS solution. The film thickness reduced to ca. 75% after immersion for 400 min. In a similar trend, the film deposited in the 10 ms/210 ms duty cycle with 200 W peak power was also unstable. In contrast, in a high duty cycle of 10 ms/50 ms and 100 W peak power, the kinetic spectra showed no or a negligible decrease during the whole immersion process. With a much higher duty cycle but the same average incident power as Figure 3a shows (10 ms/30 ms, 60 W peak power, or 10 ms/20 ms, 40 W peak power), the polymers were remarkably stable even deposited in the pulsed plasma mode.

FTIR spectra in Figure 3b and the derived data in Table 1 reveal that the plasma modes also dominated the film surface structure, especially the amine density on the surface. In the peak region at 3360 cm^{-1} , the assignment to $-NH_2$ stretching increased inversely with the decreasing duty cycle: the peak at 3360 cm^{-1} and the I_{NH_2}/I_{CH_3} ratio were lowest in the CW condition and highest in 1 ms/50 ms duty cycle pulsed plasma.

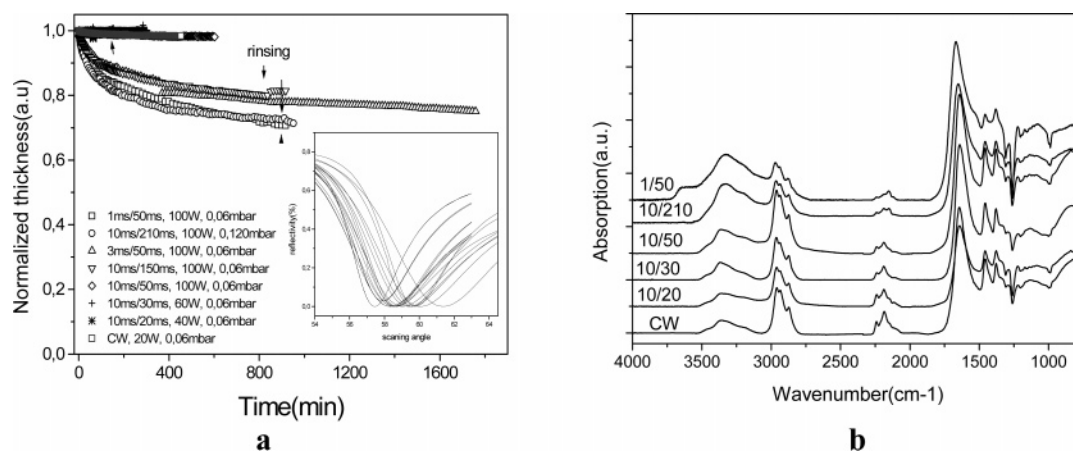


Figure 3. The relationship of polymer stability with the plasma modes and pulsed plasma duty cycles: (a) SPR spectra monitored in kinetic and scanning angle modes (the insert figure is the whole view) and (b) FTIR characterization of the plasma deposited film surface functionality in 0.06 mbar working pressure.

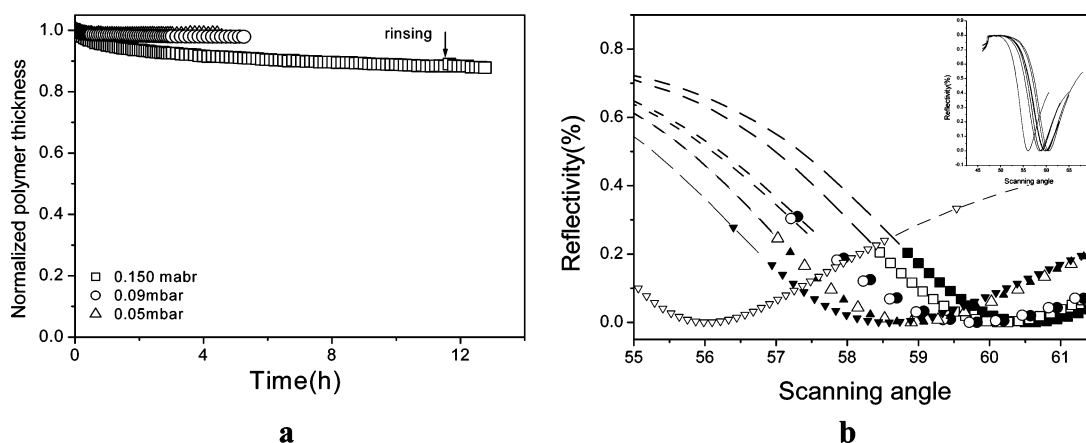


Figure 4. SPR spectra of the plasma deposited film in (a) kinetic mode and (b) scanning angle mode (insert figure is the whole view) to evaluate the influence of the plasma working pressure.

Stable does not mean nonvariable. SPR analysis in Figure 3a indicates the films being stabilized seemingly from the conditions of 10 ms/50 ms duty cycle and 20 W average power. However, a close investigation found the surface chemical structure in fact was still in variation: the amine and nitrile densities altered with the duty cycle as Table 1 shows. In the low duty cycle the peak around 2200 cm^{-1} for nitrile appeared at 2153 cm^{-1} . But with a higher duty cycle the peak moved to the high frequency, the main peak appearing at 2240 cm^{-1} with a shoulder peak at 2185 cm^{-1} , and both peaks increased with the increasing duty cycles. The explanation is that the relatively long plasma-on time t_{on} , such as 10 ms/20 ms and 10 ms/30 ms, caused a long period of electron, ion, and radical interaction, wherein a high density of cross-linking and high bonding were formed on the surface, and the polymer stability was improved. Moreover, in such plasma-on duration, when t_{off} times were extending, the films still prefers to the amine group growing on the surface as the table shows. It can be concluded that in the pulsed plasma the stabilization of the film and a variable functional group density can be achieved at the same time.

The influence of plasma working pressure on the polymer stability was also identified by the sensitive SPR technology. Figure 4 indicates the film stabilities were apparently varying with working pressures from 0.05 mabr and 0.09 mar to 0.15 mbar when deposited in 200 W peak power, CW plasma. With a 0.09 mbar working pressure, Figure 4a shows the film is relatively stable. But at a lower working pressure, i.e., 0.05 mbar, the reflectivity was still slightly down. The dip angle shifted

ca. 0.05° to the left side after immersion for 4 h as Figure 4b shows. Simulating by Frensel formulation the thickness was reduced 0.2 nm (assuming the polymer refractive index of 1.458^{14}).

In selecting monomers, SPR again plays an important role. It is known that besides allylamine monomer there are many precursors that can be employed to deposit the film with the amine group. But the capability of the monomer polymerizing in plasma, the film stabilization, and an appropriate density of functional groups on the surface are crucial parameters for the candidate monomers. In this experiment, the monomers ethylenediamine, allylamine, and allylamine hybridized with ammonia (3:2 volume) were investigated. Figure 5a shows clearly that the monomers ethylenediamine and allylamine hybridized with ammonia (3:2 v/v) were not suitable for depositing an adhesive layer due to the unstable and serious dissociation after immersion in PBS solution.

Figure 5a shows the polymer stability was poor when film was deposited by monomer ethylenediamine or allylamine hybridized with ammonia. And the amine density did not obviously increase at the 3360 cm^{-1} peak in monomer allylamine hybridized with ammonia. Especially with the monomer ethylenediamine the peak almost disappeared as seen in Figure 5b. It even assumes that the contribution of the C=C double bond in the ethylenediamine chain was not efficient enough to offer cross-linking knots as done in monomer allylamine during pulsed plasma polymerization, not only the visual instability of polyethylenedimine in SPR spectra, but also the variation of

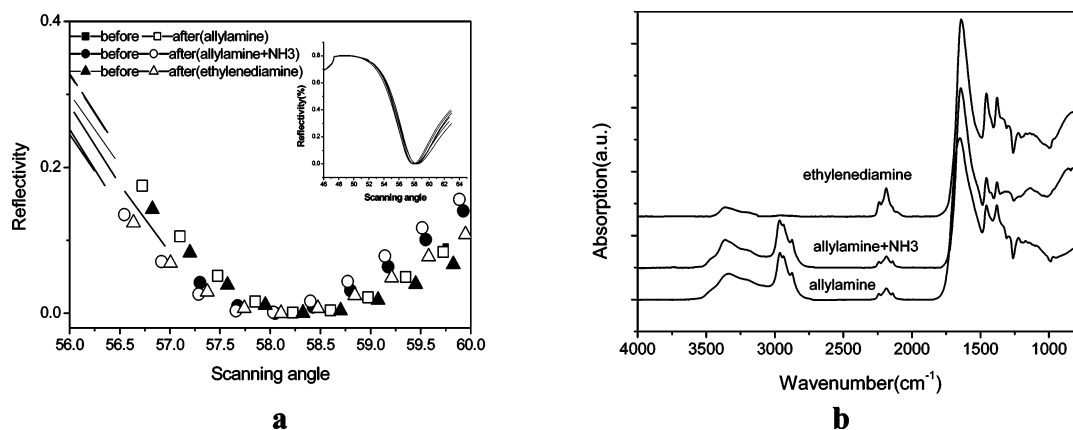


Figure 5. The influence of monomers on the stability of plasma deposited film (a) monitored by SPR in scanning angle measurement (inserted figure is the whole view) and (b) FTIR characterization of the surface functional groups, 20 W average power, 0.06 mbar working pressure.

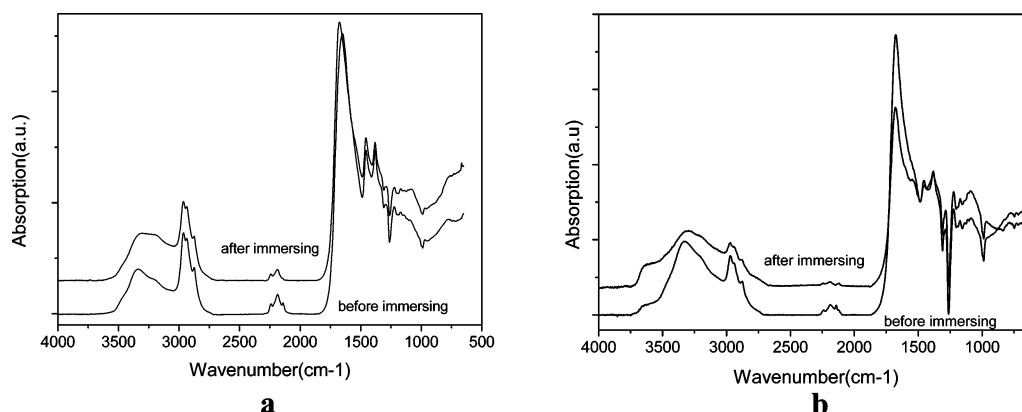


Figure 6. The influence of pulsed plasma duty cycle on the chemical structure: FTIR spectra of polyallylamine deposited in low-pressure pulsed plasma before and after immersing in PBS solution for 24 h—(a) 10/50 ms/ms and (b) 1/50 ms/ms.

the peak broad around 3360 cm^{-1} in FTIR. This suggests that a major part of the nitrogen containing functional groups may be present in a form other than -NH_2 groups in the film. The much higher peak in 2185 cm^{-1} assigned to nitrile groups in polyethylenediamine than that in polyallylamine in Figure 5b means -CN and not -NH_2 formed on the surface.

Discussion

SPR technology has been considered as a much more surface-sensitive optical spectroscopic method to characterize DNA hybridization. Now it demonstrates the ability in the ultrathin film stability research. The results show, in this experiment, that by SPR the variation of films can be monitored clearly even though it is much smaller. For example, for the film polymerized at 200 W peak power, CW plasma, and 0.09 mbar working pressure the kinetic curve illustrated that the film was stable: the reflective intensity has a negligible decay in the whole immersing process in Figure 4a. But SPR scanning spectroscopy in Figure 4b revealed the dip angle still shifted 0.05° to the left, i.e., 0.2 nm film reduced after immersion.

FTIR spectra supported the clear, reliable, and realizable SPR data with the variation of functional group density and the chemical nature before and after immersion in PBS solution. Figure 6 clarifies that a stable polymer was achieved by cross-linked surface and the CW mode or low working pressure seems to be a more efficient method to polymerize a stable film. In Figure 6a the film deposited in the 10 ms/50 ms duty cycle, 0.06 mbar low working pressure, and 100 W peak power has a negligible variation after immersion in PBS solution over 24 h, except for the wider peak around 3360 cm^{-1} attributed to the

formation of the hydrogen bond between the amine groups and aqueous solution. In contrast, the film polymerized in low duty cycle or high pressure with a lower density of cross-linked material on the surface was unstable and the film chemical structure altered after immersion in PBS solution as Figure 6b shows. The main peaks at 3360 and 1644 cm^{-1} significantly decreased and deformed, and the peak at 2185 cm^{-1} even disappeared.

AFM phase images in Figure 7b,d confirm the formation of a relatively more dense cross-linking surface in CW mode or low-pressure plasma, which is essential for the film stability: the surface was impacted in the low-pressure plasma condition. However, it is also noted that the plasma etching behavior in the CW plasma was more serious after comparing the AFM height image in Figure 7a with that in Figure 7c: the roughness was larger in the CW plasma than in pulsed plasma despite the visual peaks and valleys that appeared on both surfaces.

Moreover, combining the SPR with FTIR can even unveil that the film swelling and extracting behaviors occurred in aqueous solution simultaneously. In Figure 6b FTIR spectra demonstrate the continuous dissociation reaction of film deposited in 1 ms/50 ms duty cycle, 200 W peak power, and 0.06 mbar working pressure, which is relevant to significantly reducing the height at the 1644 cm^{-1} peak. But the variation in the 2185 cm^{-1} peak and the deformation in 3360 cm^{-1} were assigned to the chemical nature change, i.e., related to film swelling. In Figure 3 the SPR spectra show the decrease of the reflectivity in the kinetic curve and the shift of the dig angle in the scanning curve. It proves the polymer extraction in PBS solution.

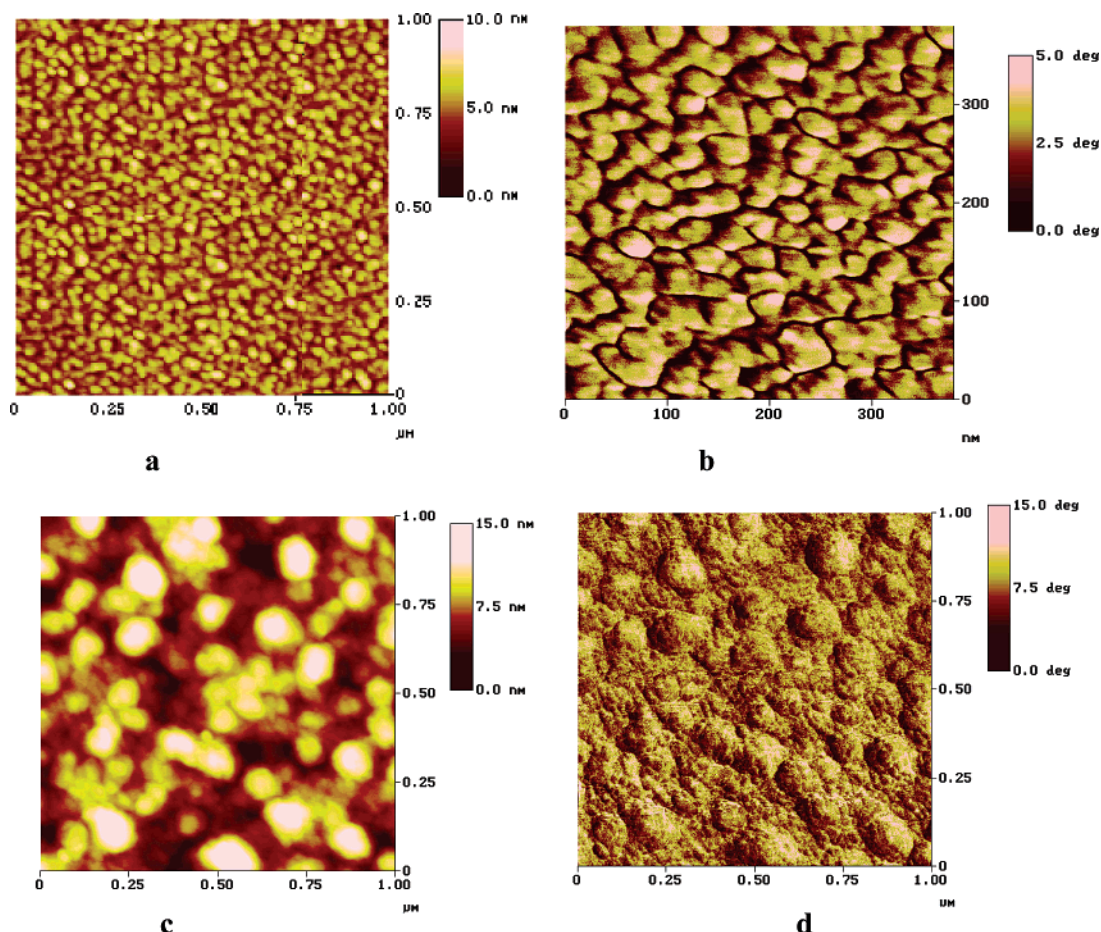


Figure 7. AFM height and phase images of plasma polymerized polyallylamine with different plasma modes: (a, b) pulsed plasma and (c, d) CW plasma.

Through precise SPR measurement the plasma polymerization mechanism can also be estimated. It is known that a high deposition rate and a high density of functional groups are obtained downstream. The continuous significant decrease of the reflectivity signal in this experiment while the film was polymerized downstream suggests the dissociation reaction of the film was caused from the aggregated oligomers leaving the surface, i.e., it is the oligomer reaction mechanism downstream by the long life, high concentration radical reactions.

Conclusion

Due to the high sensitivity, SPR was employed as an efficient method to characterize the ultrathin film stability in this experiment. It was noticed that the film stability in aqueous solution strongly depended on the monomer, plasma mode, and substrate location as well as plasma conditions. By SPR and FTIR spectra, it was determined that the film swelling and extracting behaviors occurred in the solution at the same time. It is concluded that the film polymerized in 10 ms/50 ms duty cycle, 20 W average power, and 0.06 mbar low working pressure pulsed plasma with the appropriate density of amine group is extremely stable in PBS solution and can be used to replace the still expensive and time-wasting biotinylated streptavidin as an adhesive layer for DNA hybridization assay.

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