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Surface Modification via Plasma Polymerization of Allylamine for Antibody Immobilization

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Summary: Continuous wave and pulsed plasma polymerization coatings of allylamine were investigated for antibody immobilization as a function of plasma power, monomer pressure and treatment time, and duty cycle. Conditions were optimized by evaluating the surface amine density of plasma polymer coated samples before and after aging in dry ethanol for 3 hrs. In addition, plasma polymer coatings were characterized by contact angle analyzer, α-step, and FT-IR/ATR. The continuous wave plasma polymerization provided amine density of 4.8 molecules/nm^2 at the optimum condition of 20 W, 1 min, and 60 mTorr, while the pulsed plasma polymerization coating resulted in further increased amine density to 5.2 molecules/nm^2 at the duty cycle of 60/100.

Keywords: allylamine; amine density; biosensor; plasma poymerization

Introduction

Biosensors are designed to detect and/or quantify molecules of interest in our lives such as those for medical use and environmental monitoring.[1] However, only few biosensors have been successful to date, owing to their low sensitivity, poor selectivity, slow reaction time and/or instability. This poor performance is directly related to sensor components that detect physicochemical changes upon antibody-antigen interaction. Thus, in order to enhance the performance of biosensors, antibodies have to be firmly immobilized on the transducer surface and also have good chemical interaction with antigens.[2]

Three immobilization methods have been reported, including physical adsorption, covalent bonding, and biospecific interactions.[3,4] Among these, the covalent bonding method is known to be superior over others because of its strong chemical bond formation. However, this is not easy to achieve since it requires proper functional groups on the transducer surface, as well as in the antibody, making surface modification essential if proper functional groups are to be introduced to the transducer surface. To date, the following surface modification techniques have been introduced: polymer coating, polymer/oligomer grafting, silane coupling agent coating, self-assembled monolayer (SAM), plasma etching and plasma polymerization coating.[4–9]

Among them, the plasma polymerization technique has received great attention because of its several advantages; wide selection of monomers, very thin but pinhole free film, dry process, and environmentally friendly technique.[10] A number of monomers have been utilized in plasma polymerization for biosensor application, but those containing amine moiety are preferred because of their high reactivity. A high amine density of 12 and 5.4 molecules/nm^2 was reported with ethylenediamine and allylamine, respectively.[8,9] Plasma polymerization conditions, however, were not fully optimized in terms of stability of

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plasma polymer films, which may be a critical factor in the long-term durability of biosensors.

In this study, therefore, continuous wave plasma polymerization coating of allylamine was studied as a function of plasma power, monomer pressure, and treatment time. In addition, pulsed plasma polymerization was also attempted by varying the duty cycle. The conditions were optimized by measuring the surface amine density (with 4-NBA) before and after aging in dry ethanol for 3 hrs. Plasma polymer coatings were then characterized by contact angle analyzer, α-step, and FT-IR/ATR.

Experimental Part

Materials

Slide glass (18 × 18 mm, Marienfeld, Germany) was sonicated in acetone for 15 min, followed by vacuum drying for 30 min at RT prior to plasma polymerization coating. Anhydrous ethanol (99.5%), 4-nitrobenzaldehyde (98%) and allylamine (97%) were obtained from Sigma-Aldrich.

Plasma Polymerization Coating

A RF (13.56 MHz) plasma generator (HPPS-300, Hanatek Co., Korea), consisting of a bell-jar type Pyrex chamber was used for plasma polymerization coating, as previously reported.[11] After cleaning the Pyrex chamber by O_2 plasma etching, a slide glass was staged in the center of the chamber, and the chamber was vacuumed to 1 mTorr and flushed with allylamine monomer vapor for 2 min. Plasma polymerization coating was carried out at RT as a function of plasma power (10–30 W), monomer pressure (30–70 mTorr), and treatment time(1–5 min), while pulsed plasma polymerization was studied by varying the duty cycle (800/100–20/100) with t_{on} value of 80 *m*s at the optimum conditions of continuous plasma (20 W, 1 min and 60 mTorr). In order to evaluate the stability of plasma polymer coatings, the coated samples were aged in dry ethanol for 3 hrs at 100 rpm of shaking.

Surface Amine Density of Plasma Polymer Coating

The surface amine density of plasma polymer coatings was evaluated with 4-NBA via UV- spectrophotometer.[8] Plasma polymer coated samples were immersed in 4-nitrobezaldehyde solution (10 mg in 25 ml of anhydrous ethanol) for 6 hrs at 60 °C. After thoroughly washing with ethanol and drying under vacuum at RT for 1 hr, hydrolysis of 4-NBA was carried out by immersing the samples in 4 ml of DI water at 60 °C for 12 hrs. Next, the hydrolyzed 4-NBA solution was analyzed by UV spectrophotometer (Varian, Cary 1E). All spectra were recorded after baseline correction and converted to surface amine density based on the calibration data.

Characterization of Plasma Polymer Coatings

Plasma polymer coatings were evaluated by contact angle analyser, α-step 500, and FT-IR/ATR. Contact angles were measured with 10 μl of double distilled water by static contact angle analyzer (CA-S150, Kyowa) before and after aging in ethanol for 3 hrs. Five measurements, at minimum, were made and the results were averaged. The coating thickness was analyzed by α-step 500 (Tencor) with a scan length of 100 μm at a scan speed of 10 μm/s, after scraping off a part of the coating with a razor blade. FT-IR/ATR (460, Jasco, Japan) was utilized to characterize the plasma polymer coatings (64 scans at a resolution of 4 cm^{-1}).

Results and Discussions

Plasma Power Optimization

As plasma power increased, the amine density of allylamine plasma polymer coating decreased from 4.6 (10 W) to 4.1 (20 W) and 3.6 (30 W) molecules/nm^2 at the fixed condition of 30 mTorr and 1 min (Figure 1). As reported previously,[12] higher plasma power dissociated more monomers than low power, leading to lower amine density. However, after aging the samples in dry

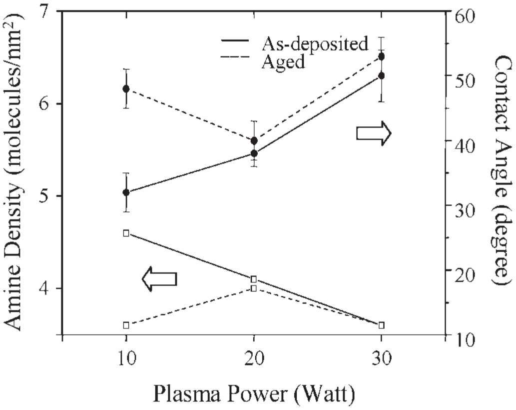

Figure 1.
Optimization of plasma power of continuous wave plasma polymerization at 30 mTorr and 1 min.

ethanol for 3 hrs, amine density increased from 3.5 (10 W) to 4.0 (20 W) and then decreased to 3.6 (30 W), providing a maximum value at 20 W. Water contact angles also followed the same trend as the plasma power (Figure 1).

It is noted that the amine density before aging was either much smaller than (10 W) or similar to (20 and 30 W) those after immersion. The similar values obtained can be attributed to the fact that the power was high enough to dissociate all monomers at 20 and 30 W, thus resulting in the formation of stable (lightly crosslinked) plasma polymer coatings. On the other hand, the smaller density obtained can be attributed to the power (10 W) being too low to dissociate all monomers to form a stable plasma polymer coating. Considering the amine density of plasma polymer films before and after aging in ethanol, 20 W was chosen as the optimum plasma power.

Monomer Pressure

As the monomer pressure increased from 30 to 70 mTorr at 20 W and 1 min, amine density increased from 4.1 (30 mTorr) to 5.0 (70 mTorr) molecules/nm^2, as shown in Figure 2. The increased amine density can be explained by less dissociation of

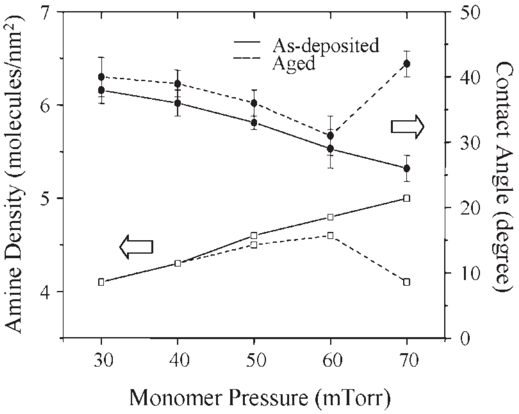

Figure 2.
Optimization of monomer pressure of continuous wave plasma polymerization at 20 W and 1 min.

Table 1.
Optimization of continuous wave plasma polymerization time at 20 W and 60 mTorr.

	1 min		3 min		5 min	
	As-dep.	Aged	As-dep.	Aged	As-dep.	Aged
Amine density (molecules/nm^2)	4.8	4.6	4.8	4.6	4.7	4.6
Contact Angle (degree)	29 ± 3	31 ± 3	30 ± 2	31 ± 3	31 ± 3	32 ± 4
Film Thickness (nm)	16 ± 0.9	–	31 ± 3.4	–	50 ± 1.7	–

monomers, owing to the decreased plasma power for each molecule as the monomer pressure increased.[13]

Upon aging in ethanol for 3 hrs, amine density exhibited an increasing trend up to 60 mTorr and then decreased at 70 mTorr (Figure 2). This behavior can be explained by the fact that 20 W was high enough to dissociate all monomers to form a stable polymer coating up to 60 mTorr, but it was not sufficient at 70 mTorr, which resulted in the formation of some oligomer type polymers. Water contact angles of samples (before and after immersion in ethanol) also exhibited the same trend as that of amine density. Therefore, the optimum monomer pressure was decided as 60 mTorr for this plasma polymerization.

Treatment Time

Plasma polymerization time did not significantly affect the amine density of plasma polymer coating and thus water contact angles (Table 1). As expected, however, the film thickness increased almost linearly from 15 nm (1 min) to 50 nm (5 min) with increasing plasma polymerization time, as shown in Table 1. Considering that thinner coatings are better for biosensor sensitivity, 1 min of plasma polymerization time was decided as the optimum treatment time, providing the optimum conditions of 20 W, 60 mTorr, and 1 min.

Pulsed Plasma Polymerization

As shown in Figure 3, the pulsed plasma polymerization further increased amine density from 4.8 to 5.7 molecules/nm^2, which is slightly higher than 5.4 reported by Kim et al.[8] and much lower than the value (12) reported by Puleo.[9] The increase can be explained by the increased off-time, which allows dissociated monomers to react with each other instead of continuing to dissociate.[14] Water contact angles also decreased as the on-time decreased, suggesting more amine moieties (Figure 3). The increased amine density by

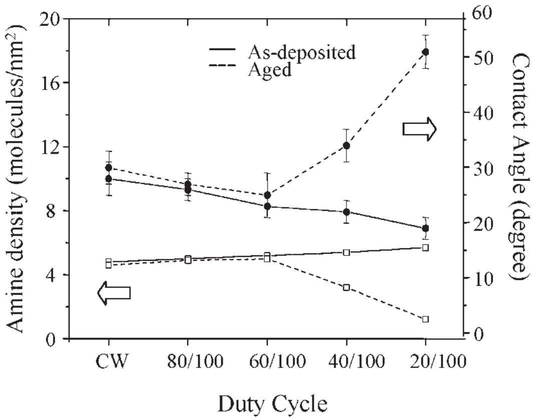

Figure 3.
Optimization of duty cycle in the pulsed plasma polymerization at 20 W, 60 mTorr and 1 min.

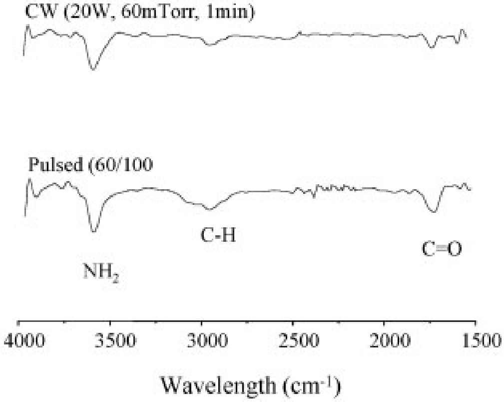

Figure 4.
FT-IR of continuous wave (at 20 W, 60 mTorr and 1 min) and pulsed (60/100) plasma polymerization coatings.

pulsed plasma polymerization is also supported by FT-IR/ATR spectra, which showed much stronger amine peak from the samples prepared at the duty cycle of 60/100, compared to that from continuous plasma polymerization (20 W, 60 mTorr and 1 min), as shown in Figure 4.

Upon aging in ethanol, the samples from 80/100 and 60/100 exhibited almost no changes in amine density, compared to those from 40/100 and 20/100, which showed dramatically decreased amine density (Figure 3). Considering the peak power, the duty cycles of 80/100 and 60/100 provided high enough power to form stable polymer films, while 40/100 and 20/100 cycles were not sufficient and resulted in unstable polymer films. Therefore, considering all the above factors including stability in ethanol, the 60/100 duty cycle was selected as the optimum condition at 20 W, 60 mTorr, and 1 min, which provided the amine density of 5.2 molecules/nm^2.

Conclusions

1. Plasma polymerization coating of allylamine was investigated as a function of plasma power (10–30 W), monomer pressure (30–70 mTorr), and treatment time (1–5 min) by measuring amine density.
2. Based on the amine density before and after aging in ethanol for 3 hrs, the optimum conditions for continuous wave plasma polymerization were selected as 20 W, 60 mTorr and 1 min, which resulted in the amine density of 4.8 molecules/nm^2.
3. Pulsed plasma polymerization further increased amine density to 5.7 molecules/nm^2 at 20/100 (unaged) and 5.2 molecules/nm^2 at 60/100 (aged), but the latter was chosen as the optimum duty cycle.
4. Considering the stability of allylamine plasma polymer coatings in ethanol, the optimum conditions are 20 W, 60 mTorr, 1 min, and 60/100, which provide the amine density of 5.2 molecules/nm^2.

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[1] B. R. Eggins, *Chemical Sensors and Biosensors*, Wiley **2002**.
[2] H. Mugurama, I. Karube, *Trends in Analytical Chemistry* **1999**, *18*, 62.
[3] K. Nakanishi, T. Sakiyama, K. Imamura, *J. Biosci. Bioeng.* **2001**, *91*, 233.
[4] J. K. Kim, D. S. Shin, W. J. Chung, K. H. Jang, K. N. Lee, Y. K. Kim, Y. S. Lee, *Colloids Surf., B* **2004**, *33*, 67.

[5] B. Gupta, C. Plummer, I. Bisson, P. Frey, J. Hilborn, *Biomaterials* **2002**, *23*, 863.
[6] W. Liao, F. Wei, M. X. Qian, X. S. Zhao, *Sensors and Actuators B* **2004**, *101*, 361.
[7] E. Ostuni, L. Yan, G. M. Whitesides, *Colloids Surf., B* **1999**, *15*, 3.
[8] J. Kim, H. K. Shon, D. Jung, D. W. Moon, S. Y. Han, T. G. Lee, *Anal. Chem.* **2005**, *77*, 4137.
[9] D. A. Puleo, R. A. Kissling, M. S. Sheu, *Biomaterials* **2002**, *23*, 2079.
[10] H. Yasuda, *Plasma Polymerization*, Academic Press **1985**.
[11] H. M. Kang, N. I. Kim, T. H. Yoon, *J. Adhes. Sci. Technol.* **2002**, *16*, 1825.
[12] M. Lejeune, F. Bretagnol, G. Ceccone, P. Colpo, F. Rossi, *Surf. Coat. Technol.* **2006**, *200*, 5902.
[13] Q. Chen, R. Forch, W. Knoll, *Chem. Mater.* **2004**, *16*, 614.
[14] M. T. Van Os, B. Menges, R. Foerch, G. J. Vansco, W. Knoll, *Chem. Mater.* **1999**, *11*, 3252.