Patterned Amine Surfaces with Reduced Background Nonspecific Protein Adsorption Fabricated by Using Inductively Coupled Plasma Chemical Vapor Deposition

Sanghak Yeo, Changrok Choi, Jaeyoung Yang and Donggeun Jung*
Department of Physics, Brain Korea 21 Physics Research Division and Institute of Basic Science, Sungkyunkwan University, Suwon 440-746

Heonyong Park
Institute of Nanosensor and Biotechnology, Dankook University, Seoul 140-714

Jin-Hyo Boo
Department of Chemistry, Sungkyunkwan University, Suwon 440-746

(Received 13 November 2006)

Nonspecific adsorption to the surface of slides decreases the sensitivity for chip-based biological assays. To solve this problem, we constructed novel patterned slides of plasma polymerized ethylenediamine (PPEDA) with protein-binding amine functional groups and a hydrophilic surface and of plasma polymerized cyclohexane (PPCHex) with a hydrophobic surface and a reduced nonspecific protein adsorption. PPEDA and PPCHex were deposited by using inductively coupled plasma chemical vapor deposition (ICP-CVD) with ethylenediamine (EDA) and cyclohexane (CHex) as precursors. PPEDA was deposited in a patterned manner on a PPCHex slide by using plasma polymerization with a patterned mask. Comparing the sample of the PPEDA/PPCHex pattern with the sample of PPEDA only, i.e., the sample with only PPEDA spots formed on bare glass slides, the average signal to noise ratio, defined as the ratio of the fluorescence intensity of the PPEDA-deposited circular spots to the fluorescence intensity of the surrounding areas, was higher for the sample with the PPEDA/PPCHex pattern, indicating that the nonspecific adsorption was reduced at the surface of PPCHex. It is thought that on the surface of PPCHex films, the reduction in the protein adsorption was more influenced by the chemical groups of the films, such as -OH groups, rather than by the physical properties of the surfaces, such as the roughness.

PACS numbers: 82.40.Np, 82.70.Uv, 87.14.Ee
Keywords: Plasma polymer, Protein, Protein adsorption, Hydrophilicity, Contact angle, AFM (atomic force microscopy)

I. INTRODUCTION

To achieve better sensitivity of diagnosis with protein chips, a sufficient mass of proteins must be immobilized to a limited area on the surface of the protein chip. The controlled immobilization for patterned arrays of biomolecules has an important implication in a wide variety of research areas, including biochips, bioelectronics, and fundamental studies of cell biology. As the requirement for patterned slides gradually increases to construct a better microarray system, several techniques, such as self-assembly and/or lithography, have been employed for the construction of the patterned slides [1]. Recently, there has been considerable research on the immobilization of biomaterials to solid slides by using a plasma polymer thin film [2–4], and even more efficient techniques for protein immobilization must be developed. The covalent immobilization of proteins depends on the chemical and/or physical entities of the glass slides coated with either nucleophilic or electrophilic functional groups. Additionally, a well-known problem associated with chip-based experiments is the nonspecific adsorption of proteins to the surface of the slides, leading to false responses in diagnostic tests. Hence, new techniques for reducing nonspecific adsorption will be more widely utilized for more efficient chip-based diagnoses [5].

A plasma, a partially ionized gas, activates molecules very effectively. Many relatively non-reactive molecules can be readily activated upon exposure to a plasma. To coat slides with plasma polymers, monomeric chemicals...
Patterned Amine Surfaces with Reduced Background Nonspecific – Sanghak Yeo et al.

are transported into the deposition chamber, activated and/or decomposed into reactive species by the plasma, and then condensed on the slides, forming thin polymer films [6]. If precursors containing functional groups, such as amine and aldehyde, are used, the surface of the plasma polymer film can contain a substantial number of these functional groups. This approach can be used to immobilize biomolecules to the solid slides. The properties of plasma polymer films are quite different from those of conventional chemically-synthesized polymer films. Plasma polymer films are pinhole free, mechanically and chemically stable, and strongly adherent to slides because of their highly cross-linked network structures [7–11]. In addition, plasma polymers can be deposited within relatively short periods of time, and with good thickness controllability and uniformity. These are great advantages for protein and DNA array preparations.

In this study, for protein array applications, we used patterned slides of plasma-polymerized ethylenediamine (PPEDA) and plasma-polymerized cyclohexane (PPCHex) deposited by using inductively coupled plasma chemical vapor deposition (ICP-CVD) with ethylenediamine (EDA) and cyclohexane (CHex) as precursors. PPEDA with hydrophilic protein-binding amine groups was used for the protein adsorption whereas PPCHex with a hydrophobic surface and, at the same time, with reduced protein adsorption was used to reduce accessibility of water and nonspecific adsorption of proteins.

II. EXPERIMENTS

1. Reagents

Ethylenediamine (EDA) and cyclohexane (CHex) were provided by Aldrich Chemical Company, Inc. 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and fluorescein isothiocyanate (FITC)-conjugated mouse immunoglobulin G (IgG) were obtained from Pierce Biotechnology, Inc.

2. Preparation of PPCHex-deposited Glass Slides

PPCHex films were deposited on glass slides (Corning microslide plain, Cat#: 2947, Corning, NY) by using ICP-CVD with cyclohexane as a precursor. The deposition system used in this work is illustrated schematically in Figure 1. The deposition chamber made of stainless steel has a cylindrical shape. The diameter and the height of the deposition chamber are 30 cm and 28 cm, respectively. The cyclohexane used as a monomeric precursor was heated to 50 °C in a bubbler. Inert Ar gas was used to carry vaporized cyclohexane molecules into the deposition chamber. The inductively coupled plasma (ICP) was generated around the shower ring by using a circular coil connected to a 13.56-MHz radio-frequency (r.f.) generator through a matching network. The slide bias (SB) power, which also generates plasma around the slide, was provided by connecting the slide holder to another r.f. generator. The deposition chamber walls were grounded. Before being loaded into the deposition chamber, the glass slides were cleaned sequentially in trichloroethylene, acetone, and methanol. The base pressure of the deposition chamber was less than ∼10−5 Torr. The deposition of PPCHex films was carried out at a slide temperature of ∼27 °C and an Ar gas flow rate of 20 sccm. The deposition pressure was kept at 200 mTorr, and the deposition time was maintained at 30 sec. The ICP power was varied from 10 W to 70 W.

3. Preparation of Patterned Glass Slides

We fabricated the patterned slides by depositing PPEDA with a patterned mask on the PPCHex/glass slide. The PPEDA films were deposited by using ICP-CVD with ethylenediamine as the precursor at room temperature and an Ar gas flow rate of 15 sccm. The deposition was performed at 30 mTorr for 2 min. The ICP power and the SB power were maintained at 4 W and 3 W, respectively.

4. Contact Angle Measurement

We carried out measurements of the static contact angles between the deposited thin films and 5 microliter of water. These measurements were based on digital images recorded by using a charge-coupled device (CCD)
mounted in the plane with the sample surfaces. The static contact angle indicated the degree of hydrophobicity or hydrophilicity of the sample surface.

5. Protein Immobilization

The EDC solution was incubated on patterned glass slides at room temperature for 10 min; then, the patterned glass slides were washed with deionized water. The solution of 4 ng/µl FITC-conjugated goat anti-rabbit immunoglobulin IgG was incubated on the patterned surfaces of the samples for 1 h. Then, the IgG solution was thoroughly washed with deionized water, and the samples were dried at room temperature.

6. Fluorescence Detection

Fluorescence from the FITC-conjugated IgG that were immobilized on the patterned slide glass was detected by using a laser fluorescence scanner. The FITC-conjugate IgG was excited at a wavelength of 488 nm, and light emission at the wavelength of 520 nm was detected.

7. Surface Morphology Investigation

We investigated the surface morphologies of the samples by using atomic force microscopy (AFM) measurement. The AFM measurements were performed in the contact mode. Scanning was performed for a 1 µm × 1 µm range.

8. FT-IR Absorption Experiment and Refractive-index Measurement

Through FTIR absorption measurements, we investigated the chemical structures and compositions of the films. The refractive indices of the thin films, which are related to film densities, were measured by using ellipsometry.

III. RESULTS AND DISCUSSION

We examined the contact angles for drops of water on the surface of all films. The contact angle of the surface of PPEDA was about 45 degree (Figure 2), indicating that the surface of PPEDA is hydrophilic. The contact angle for the surface of PPCHex was ≳ 90 degree (Figure 2), indicating the surface of PPCHex is hydrophobic. A degree of nonspecific protein adsorption to the surface of the slides was monitored by fluorescence measurements of the adsorbed proteins (Figure 3). When we dropped the protein solution on the surfaces of a bare glass slide, referred to as the control sample, and PPCHex-deposited glass slides, referred to as test samples, notable amounts of proteins were adsorbed onto the surface of the control
Patterned Amine Surfaces with Reduced Background Nonspecific

Sanghak Yeo et al.

Fig. 3. Protein fluorescence images for glass slides and PPCHex/glass slides with PPCHex deposited at various SB power. FITC-conjugate IgGs that were immobilized on the slides were detected by using a laser fluorescence scanner. FITC-conjugate IgG were excited at a wavelength of 488 nm, and the fluorescence was detected at a wavelength of 520 nm. The control sample was a bare glass slide.

Fig. 4. (a) Average signal to noise (S/N) values for the sample of PPEDA only, i.e., the sample with only PPEDA spots formed on bare glass slides, and the samples of the PPEDA/PPCHex pattern. PPEDA-covered circular spots with a diameter of 1.0 mm were formed by PPEDA deposition on the pattern mask. (b) Fluorescence images from proteins adsorbed on PPEDA/PPCHex patterned slides. Fluorescence images from two PPEDA/PPCHex glass slides with PPCHex deposited at SB powers of 10 W and 70 W are shown for clear comparison.

sample and the surface of test samples with PPCHex deposited at lower powers. As Refs. 5 and 12 show, hydrophobic surfaces do not necessarily show inhibition of nonspecific protein adsorption. The plasma polymer deposited with the hexamethydisiloxane precursor showed hydrophobic surfaces and a significant adsorption of proteins. The characteristics of the functional groups on the surfaces are thought to be more important in protein adsorption than the hydrophilicity or the hydrophobicity of the surfaces. As the deposition SB plasma power was increased from 10 W to 70 W, the nonspecific protein adsorption was reduced. Nonspecific adsorption was
minimal at a SB plasma power of 70 W. Accordingly, it can be said that one of the important parameters determining nonspecific protein adsorption is the deposition SB plasma power used during the deposition of PPCHex.

We, then, tried to fabricate patterned hydrophilic/hydrophobic surfaces. The construction for the patterned hydrophilic/hydrophobic surfaces was performed by depositing PPEDA with a patterned shadow mask placed on the PPCHex/glass slide. The PPEDA covered areas of the surface became hydrophilic, and the areas not covered by PPEDA remained hydrophobic. An ideal patterned sample of PPEDA should have had no fluorescence from the outside of the circular PPEDA spots. The ratio of the fluorescence intensity of the PPEDA-deposited circular spots to the fluorescence intensity of the surrounding areas is referred to as the signal-to-noise value. Figure 4 shows fluorescence images from slides with PPEDA circular dots. Strong fluorescence signals were emitted from those circular spots in which the amine functional groups were formed by the PPEDA deposition. The signals were much weaker from other parts of the surface that were not deposited with PPEDA. However, for some samples, there was fluorescence from areas other than the circular spots, i.e., from background areas, which was caused by nonspecific adsorption of proteins. Figure 4(a) shows the average signal to noise value for the sample of PPEDA only, i.e., the sample with only PPEDA spots formed on bare glass slides, and the samples of the PPEDA/PPCHex pattern. The surface of the bare glass slide showed a notable nonspecific adsorption of proteins, as reported previously in Ref. 13. Comparing the samples of the PPEDA/PPCHex pattern with the sample of PPEDA only, the average signal-to-noise ratio was higher for the sample with the PPEDA/PPCHex pattern, indicating that the nonspecific adsorption was reduced at the surface of PPCHex. For the samples with the PPEDA/PPCHex pattern, the background fluorescence was found to be reduced as the deposition SB plasma power used during deposition for PPCHex deposition was increased. Figure 4(b) shows fluorescence images from proteins adsorbed on PPEDA/PPCHex patterned slides. The fluorescence images from the two PPEDA/PPCHex glass slides with PPCHex deposited at SB powers of 10 W and 70 W are shown for clear comparison.

To explain the variation of the nonspecific adsorption of proteins for the PPCHex deposited at different SB powers, we studied the characteristics of the PPCHex surface. We analyzed the surface morphologies of slides by using AFM. As a function of the SB plasma power used during deposition, the root-mean-square (RMS) roughnesses of the surfaces were shown to be 0.10 nm, 0.15 nm, 0.23 nm and 0.26 nm at SB powers of 10 W, 30 W, 50 W and 70 W, respectively (Figure 5). The surface of the PPCHex film deposited at a higher plasma power was rougher than the surface of the PPCHex film deposited at a lower power. The variable RMS roughness can be explained by the exposure of the plasma to a growing film surface. The plasma exposure may then induce damage of the surface by various plasma-generated species, such as ions and charged radicals. Such plasma-induced damage is likely to increase as the plasma exposure power, in this case, the deposition power, is increased. Therefore, we think, the surface of PPCHex was roughened more severely at higher SB power. Sometimes, a rougher surface is better for protein adsorption. Previously, it was reported that proteins are more easily adsorbed on rougher surfaces [14]. However, in our case, PPCHex films deposited at higher plasma powers with rougher surfaces showed less degree of protein adsorption. It is thought that, in our work case, on the surface of PPCHex films, protein adsorption was more influenced by the chemical properties of the surfaces than by the physical properties of surfaces, such as roughness.

To study the chemical properties of the surfaces, we performed a FTIR absorption experiment. Although a FTIR absorption analysis reveals mostly the bulk properties of films, some of the chemical species in the bulk are thought to exist on the surfaces, affecting the surface chemical properties. For the PPCHex films used in the FTIR absorption experiment, the FTIR spectra were normalized to the same thickness. Figure 6 shows the FTIR absorption spectra of PPCHex films deposited at different SB powers. Depending on the plasma SB power, there was a notable difference in the chemical structure of thin film. The spectra in Figure 6 show broad peaks at 3400 cm\(^{-1}\) and at 1650 cm\(^{-1}\). The broad signals at 3400 cm\(^{-1}\) and 1650 cm\(^{-1}\) may be attributed to \(-\text{OH}\) and \(-\text{CHO}\) groups, respectively, which are caused by water vapor incorporated into the PPCHex film during the
Patterned Amine Surfaces with Reduced Background Nonspecific – Sanghak Yeo et al.

Fig. 6. FTIR absorption spectra of the PPCHex thin films deposited at various SB powers.

Fig. 7. Relative integrated peak areas of the -OH peak and the refractive index of the PPCHex thin films as functions of the plasma SB power.

PPCHex deposition and/or when, after deposition, the PPCHex films were exposed to room air. Since, in our work, the variation in the -OH intensity with a change of the SB plasma power is very obvious, we focused on -OH groups. Figure 7 shows the integrated area of the -OH peak from the FTIR spectra and the refractive index as functions of the plasma SB power. When the plasma SB power was low (10 W to 30 W), the CHex precursor was only slightly decomposed, and the film deposited at low-plasma SB power consist of species not much different from the CHex precursor. Then these films were exposed to room air, and water or oxygen was adsorbed on the surfaces of thin films, forming -OH groups [15–18]. It was reported that functional groups, such as -OH, existing on a glass surface are capable of inducing chemical adsorption of proteins [19]. However, as the plasma SB power was increased to 50 W or 70 W, the CHex precursor was much more completely decomposed and formed thin films with a structure similar to that of diamond-like carbon (DLC), which is quite inert to water vapor, not having a notable number of -OH groups. As the properties of the PPCHex film became closer to those of DLC, the refractive index will be higher, as was the case for our data in Figure 7.

In our work, although all the surfaces of the PPCHex films used in the experiments were hydrophobic, as shown by data in Figure 2, the degree of hydrophobicity is not thought to affect protein adsorption significantly. It seems that the -OH groups on the surface play an important role in protein adsorption, so reducing the -OH groups on the surface is thought to contribute to a reduction in the nonspecific adsorption of proteins.

IV. CONCLUSION

We constructed patterned slides of plasma-polymerized ethylenediamine (PPEDA) with protein-binding amine functional groups and hydrophilic surfaces and of plasma polymerized cyclohexane (PPCHex) with hydrophobic surface and reduced nonspecific protein adsorption. PPEDA and PPCHex were deposited by using inductively coupled plasma chemical vapor deposition (ICP-CVD) with ethylenediamine (EDA) and cyclohexane (CHex) as the precursors. Comparing the samples with the PPEDA/PPCHex pattern to the sample with PPEDA only, i.e., the sample with only PPEDA spots formed on bare glass slides, the average signal-to-noise ratio was higher for the sample of the PPEDA/PPCHex pattern, indicating that the nonspecific adsorption was reduced at the surface of PPCHex. For the samples with a PPEDA/PPCHex pattern, the background fluorescence was found to be reduced as the SB plasma power used during deposition for PPCHex deposition was increased. For PPCHex, the degree of hydrophobicity or the roughness of the surface did not affect the protein adsorption significantly. The -OH groups on the surface seem to play an important role in protein adsorption.

ACKNOWLEDGMENTS

This work was supported by the Science Research Center program (Center for Nanotubes and Nanostructured Composites) of Ministry of Science and Technology/Korea Science and Engineering Foundation.

REFERENCES