

Immobilization of Poly(ethylene imine) onto Polymer Films Pretreated with Plasma

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ABSTRACT: Poly(ethylene imine) (PEI)-immobilized surfaces have attracted much attention as interesting biomaterials in relation to cell culture, selective adsorption of endotoxins, and so on. In this study, we found that PEI could be effectively immobilized on various polymers pretreated by oxygen plasma. Surface analyses using an X-ray photoelectron spectrometer and contents of peroxide groups determined by iodide method indicate that the hydroxy peroxides were the main functional groups that contributed to PEI immobilization covalently on the polymer surfaces treated with oxygen plasma. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 75: 576–582, 2000

Key words: poly(ethylene imine); immobilization; surface amination

INTRODUCTION

Amino groups have been introduced onto polymer surfaces to render the surfaces more wettable,¹ cell adhesive,² and biocompatible.³ Poly(ethylene imine) (PEI) has been frequently used for the introduction of amino groups on polymer surfaces. It has been found that PEI-immobilized Sepharose⁴ and cellulose⁵ have an excellent capacity for extracorporeal adsorption of endotoxins. PEI-modified porous carriers have been shown to have many advantages over other carriers for cultivation of hybridoma in high density.⁶ Biomaterials carrying amino groups on their surfaces also have attracted much attention in the studies of nerve cell tissue culture.^{2,7} Neuronal cells were found to mature faster on poly(ethylene imine)-coated plates than those on polylysine-coated plates.⁸ In addition, amino groups

can be readily utilized for covalent binding of bioactive molecules.⁹ In most of these studies, PEI is immobilized by simple physical adsorption due to formation of multiple hydrogen bonds or polyion complexes.

PEI can be also immobilized onto the surface of hydrophobic polymers after corona¹⁰ and ozone¹¹ pretreatment. Our previous work revealed that oxygen plasma pretreatment allowed polyimide¹² and poly(methyl pentene)¹³ film to be coated with PEI. In the present study, we examine various conditions for PEI immobilization onto polymer surfaces after oxygen plasma treatment to get a deeper insight into the PEI immobilization mechanism.

MATERIALS AND METHODS

Materials

Four kinds of polymer films, polycarbonate (PC), high-density polyethylene (HDPE), poly(ethylene terephthalate) (PET), and poly(methyl pentene) (PMP), were used in this study. The PMP film was prepared from a PMP sheet (Mitsui Chemical Ltd., Tokyo, Japan) by hot pressing at 240°C to a

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thickness of about 90 μm , PC, HDPE, and PET films with thicknesses of 200, 60, and 50 μm were obtained from Toray Chemical Co., Ltd., Showa Denko Co., Ltd., and Teijin Co., Ltd., Japan, respectively. All the films were cut to a size of $1.6 \times 6.0 \text{ cm}^2$ and washed with methanol to remove surface impurities and contaminants before use. Poly(ethylene imine) (PEI, P-3143, $M_w = 60,000$) was purchased from Sigma-Aldrich Japan K. K. Co. Ltd. (Tokyo, Japan). All other chemicals were used without further purification.

PEI Immobilization

Polymer films were exposed to oxygen plasma at 0.04 Torr for predetermined periods of time, using a glow discharge reactor (LCVD 12, Shimadzu Corp., Ltd., Kyoto, Japan).¹⁴ Unless otherwise specified, the films exposed to oxygen plasma were immersed in 2 wt % PEI aqueous solution for 30 s, taken from the solution, put on a glass plate, and then baked in an oven for 3 h. After baking, the films were immersed into methanol and detached from the glass plate using an ultrasonic cleaner for 15 min. Unimmobilized PEI was removed from the films by washing with 0.1M HCl under mild shaking or with methanol using a Soxhlet extractor.

Surface Characterization

The density of peroxide groups introduced on the polymer surfaces by plasma exposure was determined with the iodide method.¹⁵ Briefly, a plasma-treated polymer film was cut into pieces and put into a glass tube that contained a mixture of 5 mL of iso-propanol, 1 mL of benzene, 0.18 mL of 0.001M ferric chloride solution in acetic acid, and 0.4 mL of sodium iodide saturated solution in iso-propanol. After nitrogen gas was introduced to replace oxygen in the solution, the tube was sealed and kept at 60°C for 1 h. The reaction was stopped by an addition of 1 mL of distilled water. The oxidized iodine was determined as triiodide anion from the absorbance of the solution at 360 nm.

The density of immobilized PEI was evaluated from uptake of an acid dye, acid orange 7.¹⁶ Amino groups on the polymer surface were complexed with the dye at pH 3, and the complexed dye was desorbed into 5 mL of 0.001M NaOH. After the pH of the solution was adjusted to acidic with an addition of 0.1 mL of 1M HCl, the absorbance at 485 nm was measured with a UV-VIS

spectrometer. The surface density of immobilized PEI was calculated under the assumption that acid orange 7 complexed with the equivalent moles of amino groups on the polymer surface.

The surface of treated films also was characterized by X-ray photoelectron spectroscopy (XPS) with an ESCA 850 instrument (Shimadzu Corp.). A magnesium-anode source producing MgK α X-ray at 8 kV and 30 mA was used, and the pressure in the instrument was maintained at 5×10^{-5} Pa throughout the analysis.

RESULTS

Surface Analyses of Oxygen Plasma-Treated HDPE

Oxygen plasma discharge is known to introduce onto polymer surface various oxygen-containing functional groups, such as alcohol, carboxylic acid, aldehyde, ketone, and peroxide.^{17,18} As an example, the C1s spectrum of HDPE exposed to oxygen plasma for 10 s is shown in Figure 1. The spectrum can be deconvoluted into four components with peak maxima at 289.1, 287.6, 286.3, and 285.2 eV assigned to O=C—O, C=O (plus O—C—O), C—O, and C—C and CH, respectively. Figure 2 further gives the O/C ratio and the relative intensity of each component after exposure of HDPE films to oxygen plasma discharge for different periods. The O/C ratio reached about 0.3 after oxygen plasma exposure for 10 s and then increased a little upon prolonged exposure. The analyses of the spectra show that the C—OH, C—OOH, or COR groups were introduced on HDPE by oxygen plasma. The density of carbonyl groups remained almost constant when HDPE was exposed to oxygen plasma discharge for 5–60 s.

The density of peroxide groups determined with the iodide method is shown in Figure 3. Obviously, the density of peroxide groups sharply increased with the plasma treatment time and then leveled off upon plasma exposure for more than 10 s. This is quite different from Ar plasma discharge.¹⁴

Optimum Condition for PEI Immobilization

Following plasma discharge for 10 s, HDPE and PET films were immersed in 2 wt % aqueous solution of PEI for 30 s, dried, and then baked at 60°C in an oven for 3 h. Both the films were subjected to ultrasonic treatment in methanol to detach the films from the glass plate. To remove

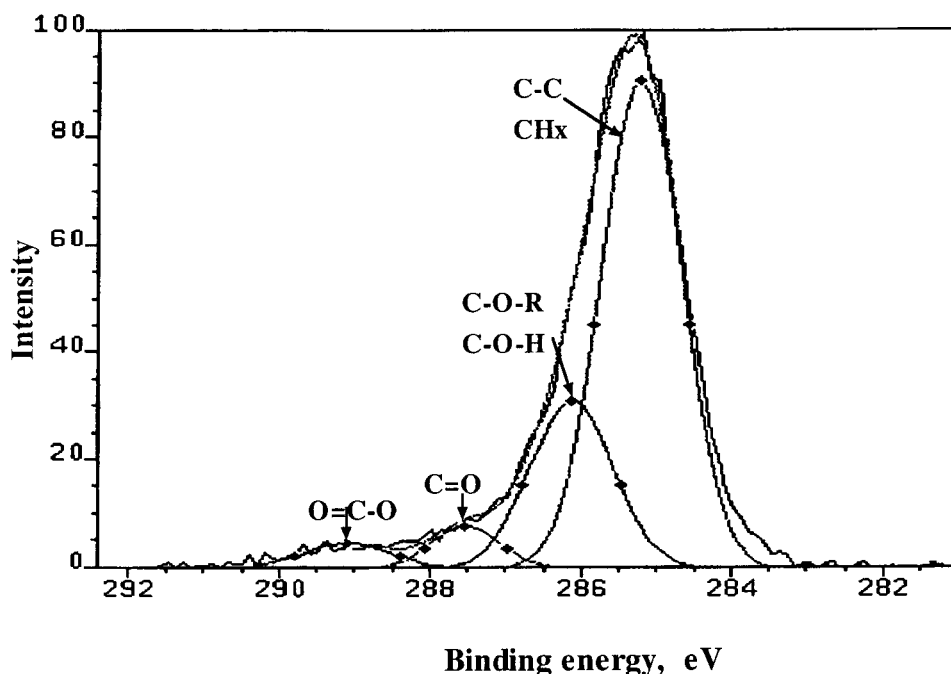


Figure 1 C1s spectrum of HDPE exposed to oxygen plasma discharge for 10 s.

the unimmobilized PEI, the PEI-bound films were extracted with methanol in a Soxhlet apparatus or immersed in 45 mL 0.1M HCl in a tube that was shaken at 100 rpm at room temperature. The HCl solution was replaced every 12 h with fresh solution. Figure 4 shows the effect of washing on the amount of immobilized PEI. The results indicate that most of the unimmobilized PEI was

removed from both the film surfaces after extraction with 0.1M HCl for 48 h or with methanol extraction for more than 24 h. In all the following experiments, samples were extracted with 0.1M HCl for 48 h to remove the unimmobilized PEI.

Figures 5–8 show the effects of pH of PEI solution, baking time, and temperature, and plasma exposure time on the density of the PEI immobilized on various polymers. For all of four polymer films, the maximum density was reached at about pH 11 (Fig. 5). At both 40°C and 60°C, the density

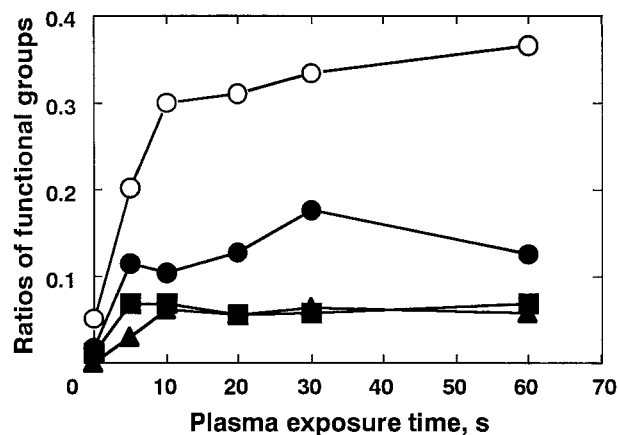


Figure 2 Oxygen/carbon atomic ratios and the relative composition of functional groups on the HDPE surface treated with oxygen plasma for various periods. O/C (○); [C—O]/C (●); [C=O]/C (■); and [O—C=O]/C (▲).

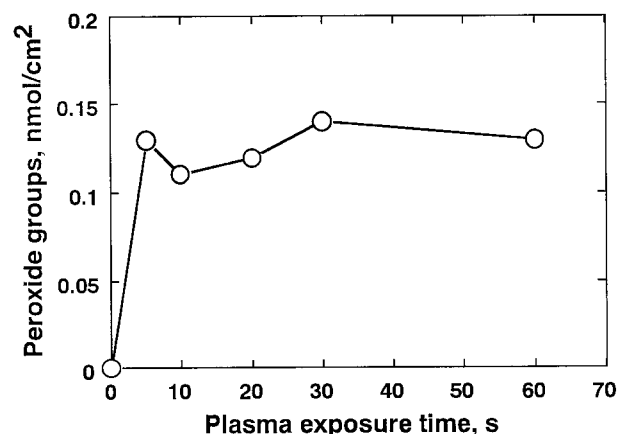


Figure 3 Peroxide density on the HDPE film treated with oxygen plasma for various periods.

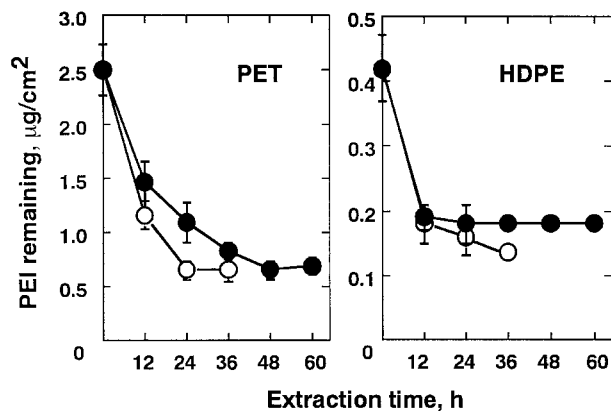


Figure 4 The effect of extraction on the density of PEI immobilized. After oxygen plasma pretreatment for 10 s, films were immersed in 2 wt % aqueous solution of PEI at pH 11 for 30 s and baked at 60°C in an oven for 3 h. The films were extracted with methanol using a Soxhlet apparatus (○) or with 0.1M HCl (●).

of the immobilized PEI increased with time during the initial 3 h and then leveled off. Prolongation of baking time did not improve the PEI immobilization (Figure 6). The maximum density of the PEI immobilized at 60°C was twice as large as that at 40°C. The temperature dependence is shown in more detail in Figure 7. It is obvious that PEI immobilization was greatly enhanced by an increase in baking temperature. Figure 8 shows that very short plasma exposure was enough for the PEI immobilization. The dependence of the PEI density on the plasma exposure

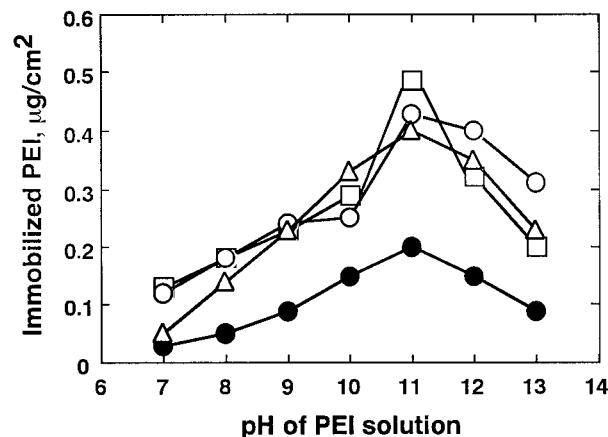


Figure 5 The effect of pH of PEI solution on the density of PEI immobilized on HDPE (●), PET (□), PC (○), and PMP (△). After exposure to oxygen plasma for 10 s, all films were immersed in 2 wt % aqueous solution of PEI for 30 s and baked at 60°C in an oven for 3 h.

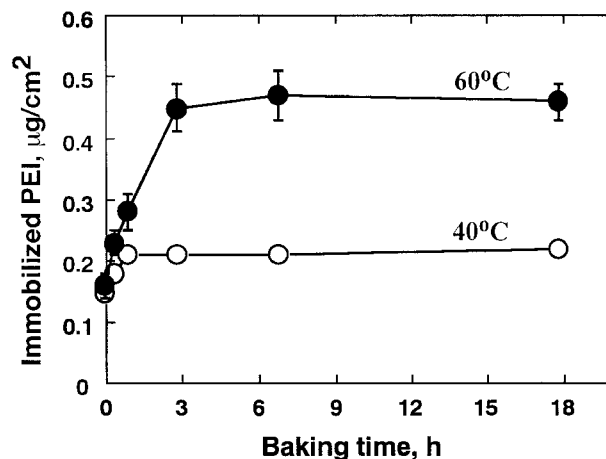


Figure 6 The effect of baking time on the density of PEI immobilized on PET when baked at 40°C (○) and 60°C (●). After exposure to oxygen plasma for 10 s, PET film was immersed in 2 wt % aqueous solution of PEI at pH 11 for 30 s and baked in an oven for 3 h.

time correlated well with that of the density of peroxide groups on the plasma exposure time.

Role of Peroxide Groups on Polymer Surface in PEI Immobilization

The results shown in Figures 3 and 8 suggest that the peroxide groups played an important role in PEI immobilization. To get a deeper insight into the mechanism of PEI immobilization, the perox-

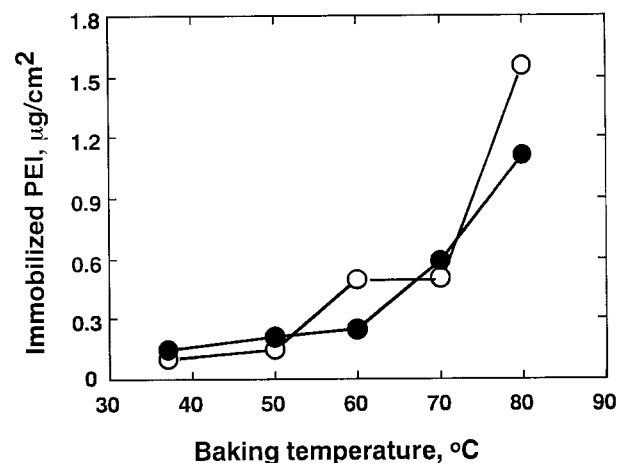


Figure 7 The effect of baking temperature on the density of PEI immobilized on HDPE and PET. After exposure to oxygen plasma for 10 s, both the films were immersed in 2 wt % aqueous solution of PEI at pH 11 for 30 s and baked in an oven at different temperatures for 3 h. HDPE (●), PET (○).

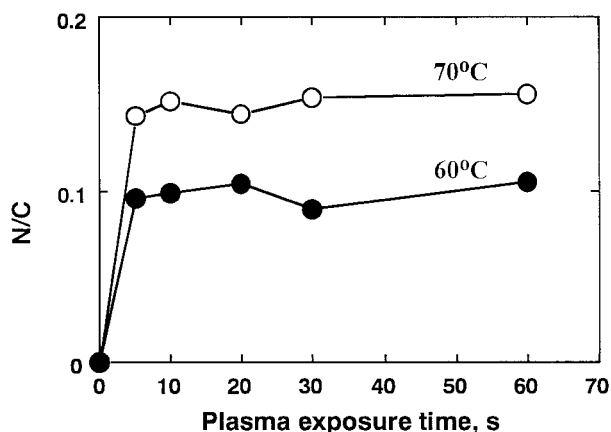


Figure 8 Nitrogen/carbon atomic ratios of PEI-immobilized HDPE surface. The HDPE film treated by oxygen plasma for various periods was immersed in the PEI solution of pH 11 for 30 s and then baked at 60°C (●) and 70°C (○).

ide groups on the plasma-treated film were decomposed using NaI, which is widely used for peroxide determination. For comparison, a similar measurement was carried out also for the plasma-treated films after immersion in the mixed solvent without NaI. Table I gives the surface atomic composition of four polymer films after exposure to oxygen plasma (treatment I) and the subsequent treatment with a mixed solvent containing with NaI (treatment II) or without NaI (treatment III). No large differences in atomic composition were observed for PET, PMP, and HDPE films between treatments I and III. However, the oxygen content significantly decreased for all the plasma-treated films when they were brought into contact with the NaI solution after plasma exposure. This significant decrease of oxygen density after peroxide decomposition with NaI suggests that peroxide groups mostly existed as ROOH (hydroperoxide groups), not as ROOR',

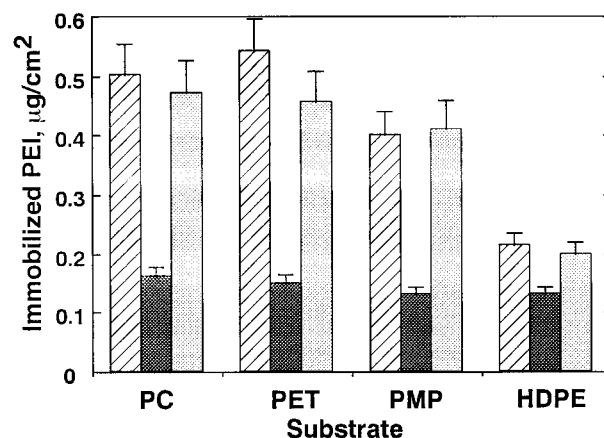
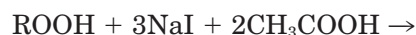
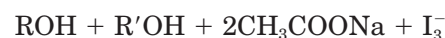
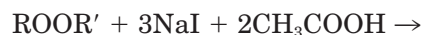


Figure 9 The effect of NaI treatment on the density of PEI immobilized on plasma-pretreated polymers. Plasma exposure alone (▨); treated with NaI solution (■); treated with solvent without NaI (□).

because decomposition of ROOR' does not give variation in oxygen density, but decomposition of ROOH decreases oxygen density as follows:

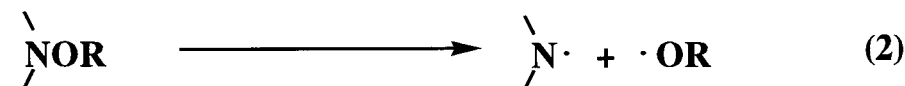
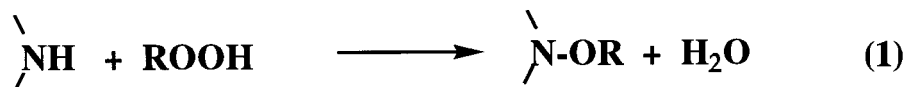
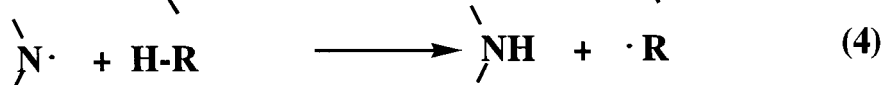
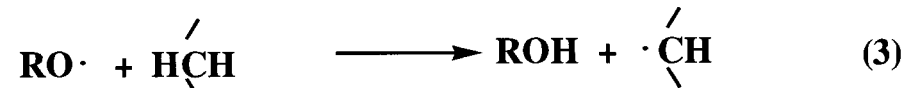
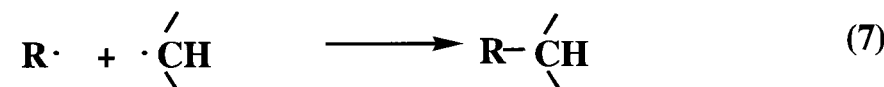
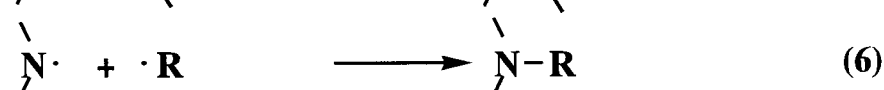
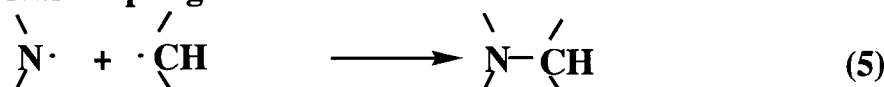


The effect of NaI treatment on the density of immobilized PEI is shown in Figure 9. The PEI density greatly decreased after decomposition of the peroxide groups on the film surface. This indicates that the hydroperoxide groups are the main reactive groups that contribute to PEI immobilization on the polymer surface treated with oxygen plasma and at the baking temperature.

Table I Surface Atomic Compositions (%) of Polymer Films after Various Treatments

Film	Virgin Sample		Treatment I		Treatment II		Treatment III	
	C	O	C	O	C	O	C	O
PC	82.89	17.11	68.09	31.91	80.83	19.17	75.12	24.88
PET	73.64	26.36	67.76	32.24	72.72	27.28	68.14	31.86
PMP	98.26	1.74	82.36	17.64	87.22	12.78	83.49	16.51
HDPE	95.43	4.57	80.44	19.76	82.09	17.91	80.77	19.23

Treatment I: O₂ plasma exposure for 10 s; treatment II: treated with NaI solution after exposure to oxygen plasma for 10 s; Treatment III: treated with a solvent mixture without NaI after exposure to oxygen plasma for 10 s.

Decomposition of peroxide groups**Radical transfer reactions****Radical coupling reactions**

R: segment of substrate polymer chains

Figure 10 Presumed scheme of PEI immobilization through peroxide formation.

DISCUSSION

Various oxygen-containing groups are introduced on the polymer surface through oxygen plasma treatment. PEI might be immobilized through several kinds of reactions, including polyion complexation, hydrogen bonding, Schiff's base formation, and radical coupling. Most of polar groups containing oxygen can readily form hydrogen bonds with the nitrogen atom in PEI molecules. The carboxylic group on the surface can form polyion complexes with the amino group of PEI molecules at a suitable pH. However, these two seem not to be the main reactions for the PEI immobilization, because they do not depend on temperature because of the very low activation energy of these reactions.

The most probable moiety responsible for the PEI immobilization are peroxides formed upon oxygen plasma exposure, because the density of the immobilized PEI drastically decreased after NaI treatment, as shown in Figure 9. It is well known that organic peroxides have been used to

crosslink both saturated and unsaturated polymers.¹⁹ Amine is generally used as a promotor to increase the rate of peroxide decomposition.²⁰ The amino groups of PEI must have promoted decomposition of the peroxides on the film surface to produce radicals.²¹ As illustrated in Figure 10, various radicals are produced on the substrate and PEI molecules through radical chain transfer reactions. The coupling reaction between the radicals should then result in the immobilization and crosslinking of PEI macromolecules. These reactions seem to account for most of the PEI immobilization on the film.

Another possible reaction that could immobilize PEI onto the plasma-treated polymer surface is formation of an amide bond through dehydration of ammonium carboxylate between a carboxylic acid on the surface and an amino group of PEI. This reaction might contribute to PEI immobilization more significantly at higher baking temperatures.

The pH dependence shown in Figure 5 indicates that some ionic species also contributed to the PEI

immobilization. We suppose that PEI molecules were first adsorbed onto the plasma-treated polymer surfaces through formation of polyion complex and then covalently immobilized or by formation of an amide bond at high temperatures.

In addition, when the baking temperature is high enough, that is, near or higher than the glass transition temperature of polymer substrates, PEI immobilization may be partly due to penetration of PEI chains into the bulk phase of the substrates.

REFERENCES

1. Favia, P.; Stendardo, M. V.; d'Agostino, R. *Plasmas Polym* 1996, 1, 91.
2. Stenger, D. A.; Pike, C. J.; Hickman, J. J.; Cotman, C. W. *Brain Res* 1993, 630, 136.
3. Terlingen, J. G. A.; Brenneisen, L. M.; Super, H. T. J.; Pijpers, A. P.; Hoffman, A. S.; Jan Feijen, J. *Biomater Sci Polym Ed* 1993, 4, 165.
4. Petsch, D.; Deckwer, W. D.; Anspach, F. B.; Legal-lais, C.; Vijayalakshmi, M. J. *Chromatogr (B) Biomed Sci Appl* 1998, 707, 121.
5. Weber, C.; Henne, B.; Loth, F.; Schoenhofen, M.; Falkenhagen, D. *ASAIO J* 1995, 41, M430.
6. Ong, C. P.; Poertner, R.; Maerkl, H.; Yamazaki, Y.; Yasuda, K.; Matsumura, M. J. *Biotechnology* 1994, 34, 259.
7. Bander, G. A.; Goslin, K. *Culturing Nerve Cells*, MIT Press: Cambridge, MA, 1991.
8. Lelong, I. H.; Petegnief, V.; Rebel, G. J. *Neurosci Res* 1992, 32, 562.
9. Girardeaux, C.; Zammattéo, N.; Art, M.; Gillon, B.; Pireaux, J. J.; Caudano, R. *Plasmas Polym* 1996, 1, 327.
10. Rice, S. W.; Rudd, D. U.S. patent 5,156,904, (1992).
11. Nojiri, C.; Kuroda, S.; Saito, N.; Park, K. D.; Hagiwara, K.; Senshu, K.; Kido, T.; Sugiyama, T.; Kijima, T.; Kim, Y. H.; Kiyotaka, S.; Tetsuzo, A. *ASAIO J* 1995, 41, M389.
12. Zhu, B.; Iwata, H.; Hirata, I.; Ikada, Y. *J. Adhesion Sci Technol*, (to appear).
13. Iwata, H.; Zhu, B.; Ko, I. K.; Endo, S.; Iizuka, M.; Nakano, T.; Nagaoka, S.; Hirano, Y.; Ikada, Y. *Biomaterials*, to appear.
14. Suzuki, M.; Kishida, A.; Iwata, H.; Ikada, Y. *Macromolecules* 1986, 19, 1804.
15. Kulik, E. A.; Ivanchenko, M. I.; Kato, K.; Sano, S.; Ikada, Y. *J. Polym Sci Part A Polym Chem* 1995, 33, 323.
16. Kato, K.; Ikada, Y. *Biotechnol Bioeng* 1996, 51, 581.
17. Nuzzo, R. G.; Smolinsky, G. *Macromolecules* 1984, 17, 1013.
18. Yao, Y.; Liu, X.; Zhu, Y. *J. Adhes Sci Technol* 1993, 7, 63.
19. Mark, H. F.; Bikales, N. M.; Overberger, C. G.; Menges, G. (Eds.) *Encyclopedia of Polymer Science and Engineering*; Wiley: New York, 1986; Vol. 5, p 399.
20. Finch, C. A. (Ed.) *Chemistry and Technology of Water-Soluble Polymers*; Plenum: New York, 1983, p 36.
21. Patai, S. (Ed.) *The Chemistry of Peroxides*; Wiley: New York, 1983, p 547.