Communication

Surface Engineering of Polystyrene Dish for Improvement of Cell Adhesion Using Plasma Techniques

Yasushi Sasai, Natsuko Matsuzaki, Shin-ichi Kondo, Yukinori Yamauchi†, and Masayuki Kuzuya†

Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502-8585, Japan
†Department of Pharmaceutical Physical Chemistry, Faculty of Pharmaceutical Science, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578 Japan
sasai@gifu-pu.ac.jp

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1. Introduction

Polystyrene (PS) has commonly been used in cell-culture dishes because of its good optical qualities, handling characteristics and nontoxic surface for cell growth. However, PS must be subjected to a surface treatment for biomedical use because it is a hydrophobic polymer. It is well-known that the suitable wetting surface with a water contact angle of about 40º can enhance the adhesion of cultured cell on the substrate surface. Furthermore, the surface chemistry on the substrate affects the cell adhesion and proliferation. [1]

We have reported the novel method to introduce a durable surface hydrophilicity and minimize its decay with time on several hydrophobic plasma-crosslinkable polymers (polyethylene-naphthalate (PEN), polyethylene, nylon-12) using plasma techniques.[2-4] This method involves a sorption of vinylmethylether-maleic anhydride copolymer (VEMA) into the surface layer and the immobilization by plasma-induced crosslink reaction, followed by hydrolysis of VEMA to generate hydrophilic carboxyl groups (VEMAC) on the surface. The conceptual procedure is sequentially illustrated in Fig. 1.

It has been known that the argon plasma irradiation to PS produces the highly crosslinked layer on the surface because cyclohexadienyl-type radicals formed in plasma-irradiated PS are strongly concerned in crosslinking. [5] Thus, we applied the method shown in Fig. 1 to PS substrate and succeeded in the introduction of carboxyl groups onto the PS substrate. [6]

In this study, we examined the optimization of the plasma conditions for the effective immobilization of VEMAC on PS dish surface. The cell adhesion and proliferation on the PS dish immobilizing VEMAC (PS/VEMAC) were compared with those on the non-treated PS and the commercial tissue culture PS (TCPS) dishes.
2. Experimental

2.1. Preparation of PS/VEMAC

A commercial polystyrene (PS) dish (35 mm in diameter) was cleaned by ultrasonication in methanol and dried in vacuo at room temperature. The washed PS dish was used as non-treated PS in this study. 1 % (w/v) VEMA solution of dimethyl sulfoxide (DMSO) was added in the PS dish and it was incubated for 1 h at 30 °C to penetrate VEMA into PS surface layer. Then, the PS dish was washed with DMSO to remove the excess VEMA on the surface. After drying at 50 ºC in vacuo for more than 10 hours, the PS dish thus treated was submitted to Ar plasma irradiation to immobilize the VEMA onto the surface layer. Figure 2 shows the experimental set-up for plasma irradiation. The plasma state was generated by the use of radio-frequency discharge of inductive coupling with five loop antenna at 13.56 MHz with the prescribed power. Flow volume (50 ml/min) and pressure (66.6 Pa (0.5 Torr)) of argon gas were controlled by evacuating speed. The sample was placed in the reaction chamber (230 mm long, 45 mm in diameter) to ensure homogeneous exposure to plasma gas. After plasma irradiation, the hydrolysis of the maleic anhydride linkage in VEMA was conducted by immersing the PS dish immobilizing VEMA (PS/VEMA) in 0.1 M NaOH solution for 10 minutes. The hydrolyzed PS/VEMA dish was soaked in 1 M HCl for 10 minutes at room temperature and washed with distilled water to obtain PS/VEMAC dish.

2.2. Surface characterization

The concentration of carboxyl group on PS surface was determined by the following method, based on the assumption that Toluidine Blue O (TBO) was complexed to equivalent moles of carboxyl group on solid surface. [7] Carboxyl groups on the PS surface were complexed with 5 × 10^{-4} M TBO solution of pH 10 at 30 °C for 5 h and non-complexed TBO was then removed with 1 × 10^{-4} M NaOH. TBO complexed to carboxyl groups on the PS surface was desorbed with 50% acetic acid solution and the absorbance of this solution at 630 nm was measured.

2.3. Cell culture

PS/VEMAC and non-treated PS dish were sterilized by UV irradiation. PC-3 human prostate cancer cell was used as a model anchorage-dependent cell for cell adhesion test. PC-3 cells were routinely cultured in RPMI-1640 medium supplemented with 10 % calf serum at 37 °C in a 5 % CO_{2} incubator. After trypsin treatment, the cell density was adjusted to 8.3 × 10^{4} cells/mL, and 2 mL of the cell suspension was seeded into PS/VEMAC dish and the control dishes (non-treated PS and TCPS (SUMILON, Sumitomo Bakelite Co., Ltd, Japan)). After a given time, the behavior of cell adhesion on the PS surfaces was observed with phase contrast microscope. The number of cells adhered on each dish was counted after 72 h in culture using hemacytometer (Burker-Turk type, Erma Inc., Japan).

3. Results and Discussion

3.1. Plasma conditions for preparation of PS/VEMAC dish

We have previously examined the optimal molecular weight of VEMA for immobilization onto PS by plasma irradiation. When VEMA with molecular weight of 4.1×10^{4} was used for immobilization, the highest density of carboxyl group was observed on PS/VEMAC surface. [6] Based on the result, VEMA with molecular weight of 4.1×10^{4} was used in this study.

In order to optimize the plasma conditions for immobilization of VEMA on PS dish, we examined the effect of plasma conditions on the density of carboxyl group on PS/VEMAC dish. Figures 3A and 3B show the effects of power and duration for plasma irradiation, respectively. As shown in Fig. 3A, a maximum density of carboxyl groups was observed on the PS/VEMAC dish prepared by plasma irradiation at 30W for 30s under the present plasma conditions. On the other hand, when the plasma duration increased, the surface density of carboxyl group increased up to 60 s and then leveled off (Fig. 3B). These results suggest that the selection of optimal plasma condition is very important for the preparation of
PS/VEMAC surface with high density of carboxyl groups and argon plasma irradiation with the higher power (over 40W in this case) results in decrease of carboxyl group on PS/VEMAC surface due to plasma-induced decomposition of VEMAC. The water contact angle of PS/VEMAC dish surface prepared by argon plasma irradiation at 30W for 30s showed about 35º and remained unchanged over a long period of time (data not shown). This result indicates that VEMAC is strongly immobilized on PS dish surface by plasma-induced cross-link reaction of PS.

3.2. Cell adhesion properties on PS/VEMAC dish

PC-3 cell suspension (1.7 ×10⁵ cells/dish) was seeded into PS/VEMAC dish and control dishes (non-treated PS and TCPS dish). Figure 4 shows the microscopic images of PC-3 cells on each dish after 6 h and 12 h in culture. As can be seen in Fig. 4, the cells adhered on non-treated PS dish are not apparently observed even after 12 h in culture. On the other hand, the cell adhesion behavior in PS/VEMAC dish was significantly improved and apparently equal to one in TCPS dish.

Figure 5 shows the number of cells adhered on each dish after 72 h in culture. The cells adhered on non-treated were about 3 % of seeded cells (5.2×10⁵ cells/dish). As can be seen in Fig. 5, a...
good cell proliferation equal to TCPS was observed on PS/VEMAC surface and the number of adhered cells was over fourfold of seeded cells, although the negative effects of surface carboxyl groups on cell adhesion have been reported for a number of cell types. [1, 8] This unexpected result might be ascribed to the suitable hydrophilicity of PS/VEMAC surface and/or a good cell compatibility of VEMAC immobilized on PS.

4. Conclusion

It can be concluded that the PS/VEMAC surface should be useful for cell culture substrate. A further study is needed to make clear the mechanism which the cell adhesion and proliferation are enhanced on PS/VEMAC surface having a large amount of carboxyl groups.

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References