

세포배양용 Polystyrene Dish의 표면개질

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Surface Modification of Polystyrene Dishes for Enhanced Cell Culture

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요약 : PS dish 표면들을 수증기 플라즈마로 방전 처리하여 수산기를 도입하고, 기존에 널리 사용되고 있는 산소 플라즈마 처리한 PS dish들 및 시판되고 있는 세포배양용 PS dish들(산소플라즈마 또는 크로마 처리)과의 표면물성 변화를 물 접촉각 측정과 ESCA에 의해 비교 분석하였다. 이들 dish들은 모두 공기중 방치시나 물 세척시 표면 경시 변화를 보이는 것이 확인되었다. CHO 세포를 이들 PS dish에 배양후 표면에 부착된 세포들의 갯수를 측정하고 부착된 양상을 SEM으로 관찰해 본 결과, 수증기 플라즈마 처리한 PS dish들 표면에서의 세포 부착성 및 퍼짐성이 산소 플라즈마 처리한 PS dish들이나 시판되고 있는 세포 배양용 PS dish들보다 우수한 것으로 나타났다. 수증기 플라즈마 처리에 의해 표면 개질시 세포 배양 용기의 고품질화에 응용 가능성이 높을 것으로 판단된다.

Abstract : Polystyrene(PS) dishes(bacteriological grade) were treated with water vapor plasma to introduce hydroxyl groups onto the surfaces. The surface properties of water vapor plasma-treated dishes were characterized by water contact angle measurement and electron spectroscopy for chemical analysis(ESCA) and compared to those of oxygen plasma-treated or commercial tissue culture grade PS dishes. The changes in surface wettability and chemical structure of the PS dishes were estimated with relation to aging in air and water washing. The adhesiveness of Chinese hamster ovary(CHO) cells on the PS dish surfaces was determined by counting the numbers of the cells attached on the surfaces with an electronic cell counter. The morphologies of CHO cells adhered and spread on the dish surfaces were also examined by a scanning electron microscope(SEM). It was observed that the water vapor plasma-treated PS dishes show better cell adhesion and spreading than the oxygen plasma-treated or commercial tissue culture grade PS dishes. The water vapor plasma treatment is probably a simple and effective method to treat PS dishes or flasks for enhanced cell culture.

INTRODUCTION

Many cell types are classified as anchorage-dependent and grow only when attached to a suitable surface. Glasses are considered suitable for the culture of many anchorage-dependent cells due to the polar groups on their surfaces. Disposable PS dishes, as substitutes of glass dishes, have been used for cell culture since about 1965.¹ Untreated PS surfaces have no polar groups and are generally unsuitable for cell culture, however, the PS must be subjected to a surface treatment to render the dishes suitable for cell attachment. Several processes have been used to make the dishes suitable for the attachment of cells; air or oxygen plasma and corona discharge treatments are now commercially used for the surface treatments of the PS dishes.²⁻⁴ These surface treatments can produce oxygen-based polar groups including hydroxyl groups, ketone groups, carboxyl groups and sometimes other groups such as ethers, aldehydes or esters.

In a previous paper,⁵ we have described the modification of various polymer surfaces such as polyethylene, polypropylene, PS, polyethylene terephthalate and poly(methyl methacrylate) by water vapor plasma discharge treatment. Upon treatment with water vapor plasma, the wettability of the polymer surfaces increased greatly and almost all functional groups produced on the surfaces were hydroxyl groups. The water vapor plasma-treated polymers showed good adhesion, spreading and growth of cells on the surfaces with high hydroxyl group density.

In this study, PS dishes(bacteriological grade) were treated with water vapor plasma to introduce hydroxyl groups onto the surfaces. The surface properties and cell adhesiveness of the water vapor plasma-treated PS dishes were compared to those of the oxygen plasma-treated or commercial tissue culture grade PS dishes.

EXPERIMENTAL

Substrates

Untreated PS dishes(bacteriological grade, sterilized; size, 60 mm×15 mm; approximate growth area, 21 cm²; purchased from Corning, USA) were used as the substrates for the surface treatments and the following cell culture experiments. Commercial tissue culture grade PS dishes of the same size(Corning, sterilized) were also used for the comparison.

Plasma Discharge Treatments

PS dishes(bacteriological grade) were treated with a custom-designed radio frequency glow discharge(RFGD) plasma generating apparatus in water vapor atmosphere(Fig. 1). The power supply of the RFGD generator was 200 V, 160 mA at 100 kHz. The plasma treatment was performed in a bell-jar type reactor chamber at 13.3 Pa vacuum. The PS dishes to be treated were located on the round electrode plate in the reactor chamber. The chamber was degassed for 10 min, then purged with water vapor for 10 min at the rate of 10 ml/min from the container immersed in a constant temperature(40 °C) water-bath. Then the dishes in the reactor chamber were exposed to the plasma for given times up to 60 sec. These conditions provided a stable H₂O plasma. After the chamber was degassed again for 10 min, the plasma-treated di-

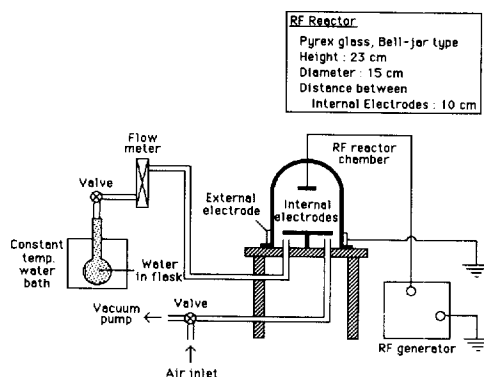


Fig. 1. Schematic diagram of water vapor plasma discharge apparatus.

shes were taken out from the chamber and used for characterization and the following cell culture experiments.

The PS dishes were also treated with oxygen plasma at the same conditions as the water vapor plasma treatment, except for purging the reactor chamber with oxygen gas instead of water vapor.

Surface Characterization

The water vapor plasma-treated dishes were characterized by the measurement of water contact angle and ESCA. The water contact angle, an indicator of wettability or hydrophilicity of surfaces, was measured at room temperature using a contact angle goniometer(model 100-0, Rame-Hart, Inc., USA). The contact angles were measured at several different points for each dish surface. For each point, 3 μ l of purified water was deposited on to the surface. At least three different dishes were measured and averaged. The water vapor plasma-treated dishes were also analyzed using ESCA (ESCALAB MK II, V. G. Scientific Co., UK) equipped with Al K α at 1487 eV and 300 W power at the anode.

The oxygen plasma-treated or commercial tissue culture grade PS dishes were also characterized in the same manner as mentioned above.

Cell Cultures

CHO cells(CHO-KI-BH₄, Oak Ridge National Laboratory, USA) were used to evaluate the effect of water vapor plasma treatment of the dish surfaces on their interaction with cells. The CHO cells are frequently used as a model system because they exist as reasonably stable single cells and are not unreasonably fastidious in terms of culture requirements.⁶ They can be grown in liquid suspension culture(anchorage-independence) and in monolayer(anchorage-dependence) with fast generation time(about 12 hr).

The CHO cells, routinely cultured in tissue culture PS flasks(Corning) at 37 °C under 5% CO₂ atmosphere, were harvested after trypsin treatment(0.05% trypsin/0.02% EDTA(Gibco Laboratories, USA)). The cells(4×10^4 cells/cm²) were seeded to the PS dishes(untreated, water vapor plasma-treated, oxygen plasma-treated and com-

mmercial tissue culture grade PS dishes) and incubated at 37 °C under 5% CO₂ atmosphere. The culture medium used was Ham's F-12 nutrient mixture(Gibco Laboratories) containing 100 units/ml penicillin and 100 μ g/ml streptomycin with 5% fetal calf serum(FCS). Further detailed procedures for the cell culture were described in previous papers.^{5,7}

The cells attached on the dish surfaces were washed with Dulbecco's phosphate buffered saline free of Ca⁺⁺ and Mg⁺⁺(PBS, pH 7.2~7.3)⁵ and trypsinized. The cell adhesion to the surfaces was determined by counting the number of attached cells with an electronic cell counter(Model ZM, Coulter, USA). The results were expressed as the percentage of cells seeded to the surfaces. The cells adhered and spread on the dish surfaces were also examined by a scanning electron microscope (SEM, JSM-840A, Jeol, Japan). After washing with Dulbecco's PBS, the cells attached on the dishes were fixed with 2.5% glutaraldehyde in PBS for 10~15 min at room temperature. After thorough washing again with PBS, the cells were dehydrated in ethanol graded series, 50%, 60%, 70%, 80%, 90% and 100%, for 10 min each and allowed to dry at room temperature. Then the cell-attached dishes were cut into small pieces, gold-deposited in vacuum and examined by SEM with the tilt angle of 45°.

RESULTS AND DISCUSSION

Surface Characterization of the PS Dishes

The water vapor plasma-treated PS dishes did not show any noticeable changes to the eye, but were wetted as water was dropped on the surfaces. The water contact angles of the surfaces treated at different water vapor plasma exposure times up to 60 sec are shown in Fig. 2. The contact angles sharply decreased with increasing plasma exposure time. However, after about 10 sec, no further significant changes were observed. The contact angle is usually considered to be a sensitive probe of changes in wettability of surfaces. The decrease in contact angles(thus, increase in wettability) with

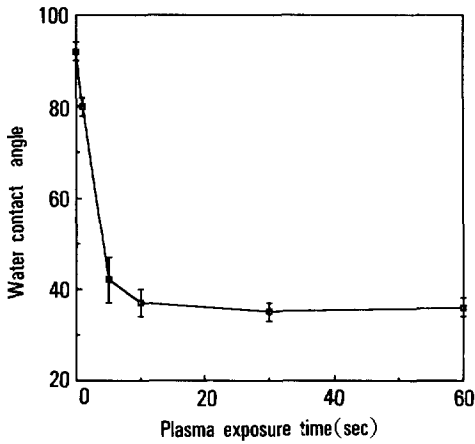


Fig. 2. Changes in water contact angle on PS dish surfaces as a function of H₂O plasma exposure time. Sample numbers, n=3.

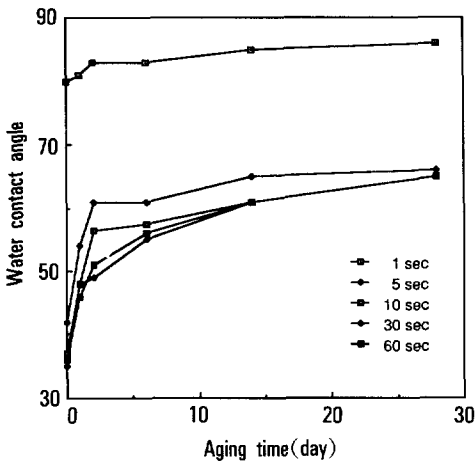


Fig. 3. Changes in water contact angle on PS dish surfaces as a function of aging time at different H₂O plasma exposure times (n=3; standard deviations $\leq \pm 3.0^\circ$).

plasma exposure is probably due to the oxygen-based polar functionalities incorporated to the surfaces; in the case of water vapor plasma treatment, hydroxyl groups are predominant on the surfaces as determined by ESCA and the labeling of the functional groups with ESCA-sensitive elements.^{5,8}

Plasma treatments are a well-known method to increase the wettability of polymer surfaces, however, it is also well known that the plasma-treated

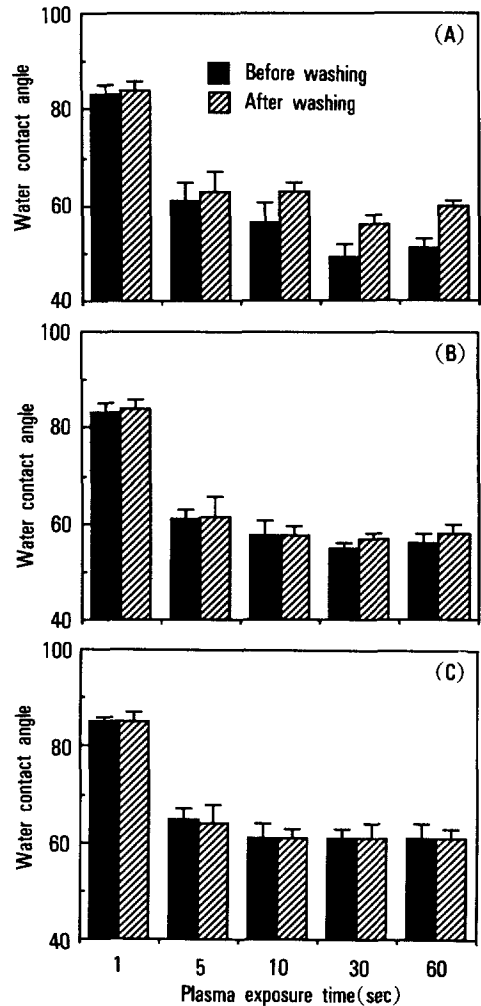


Fig. 4. Comparison of water contact angles on PS dish surfaces before and after water washing as a function of H₂O plasma exposure time at different aging times (n=3). (A) 2 day, (B) 6 day and (C) 14 day.

surfaces are subject to aging.⁹⁻¹¹ Fig. 3 shows the results of aging in air on the PS dish surfaces treated at different water vapor plasma exposure times. During the aging, the dishes were stored in a clean bench with controlled humidity (50% of relative humidity) at room temperature. The contact angles of the water vapor plasma-treated dish surfaces increased rapidly over the first 2 days, followed by slower increase. The dishes exposed for

longer time to the plasma appeared to take more time to reach a plateau. The aging, thus the increase in contact angles or the decrease in wettability, is thought to be derived from the rearrangement of the surface polar groups to their stable states or the buildup of a hydrocarbon overlayer during the storage of the dishes in air.^{9,12}

PS dishes are always in contact with the medium during cell culture and it is important to recognize whether or not the surface properties are affected as exposed to aqueous solutions. Fig. 4 shows the effect of water washing on the PS dish surfaces treated at different water vapor plasma exposure times. The plasma-treated dishes were washed with flowing distilled water for 1 min and dried in a vacuum oven. More than 1 min washing did not show any significant changes in contact angles. The dishes aged for 2 day(Fig. 4(A)) showed the increase in contact angles to some extent after water washing. The dishes aged for 14 day (Fig. 4(C)), however, did not show significant changes in contact angles, before and after the washing. It is expected that some oxygen-based polar groups incorporated to the dish surfaces by the plasma treatment were still unstable for the short time-aged dishes and were removed by water washing.^{4,11,12} For the longer time-aged(over 14 day) dishes, the surface polar groups might be sufficiently rearranged to their stable states in air and thus were little removed from the surfaces by the washing. In this study, the PS dishes aged for 14 day after the water vapor plasma treatment were used for the following cell culture experiments.

Table 1 compares the water contact angles and the surface oxygen contents(before and after the water washing) of the PS dishes used for the cell culture experiments. All the dishes used did not show big differences in the contact angle and the oxygen content after water washing. Although we could see few differences from the data of the contact angles and the ESCA survey scan spectra between the water vapor plasma-treated dishes and the oxygen plasma-treated or commercial tissue culture grade(oxygen plasma- or corona-treated) dishes, the ESCA carbon 1S core level scan spec-

tra convinced us that the functional groups produced on the surfaces are quite different ; that almost all functional groups produced on the water vapor plasma-treated dish surfaces were hydroxyl groups, while those produced on the oxygen pla-

Table 1. Comparison of the Water Contact Angles and Surface Oxygen Contents, before and after Water Washing, on the PS Dishes Used for Cell Culture Experiments(n=3)

Dish type	Water contact angle		Surface oxygen content(%) ^a	
	Before washing	After washing	Before washing	After washing
Bacteriological grade				
Untreated	93± 1	93± 1	0	0
O ₂ plasma-treated ^b	53± 3	57± 2	16.5± 0.9	15.7± 0.5
H ₂ O plasma-treated ^b	61± 2	61± 3	15.2± 0.5	14.6± 0.7
Tissue culture grade				
	60± 2	61± 2	12.4± 0.6	12.0± 0.3

^a From the analysis of ESCA survey scan spectra.

^b Plasma treatment time, 30 sec ; aging time, 14 day.

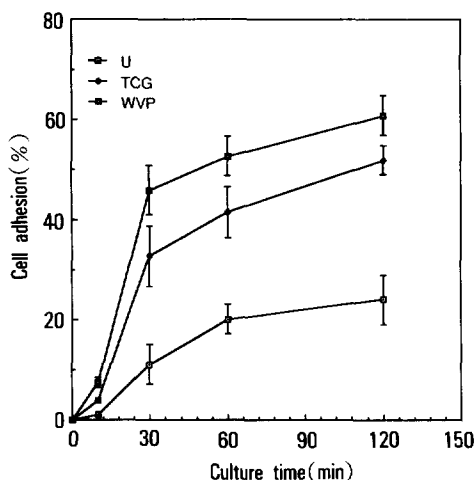


Fig. 5. Adhesion kinetics of CHO cells on PS dish surfaces as a function of culture time (medium with serum, 5% FCS ; number of seeded cells, $4 \times 10^4/cm^2$; n=3). U, untreated ; TCG, tissue culture grade ; WVP, H₂O plasma-treated PS dish surfaces.

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sma- or corona-treated surfaces were mainly hydroxyl, ketone and carboxyl groups, as discussed in previous papers.^{5,8,13}

Cell Cultures on the PS Dishes

CHO cells were cultured on the untreated, commercial tissue culture grade and water vapor plasma-treated PS dishes in the medium containing 5% FCS and the cell adhesiveness of the dishes were compared. Fig. 5 compares the adhesion kinetics of the CHO cells to the PS dish surfaces, as

counted the number of the cells adhered on the dish surfaces by an electronic cell counter. Up to 10 min culture, the cells were poorly attached to all the dish surfaces. After 10 min, however, the cells started to adhere greatly on the tissue culture grade and water vapor plasma-treated dish surfaces, while they were still poorly attached on the untreated dishes. The water vapor plasma-treated PS dishes showed best cell adhesiveness among the three dishes. Fig. 6 shows the SEM

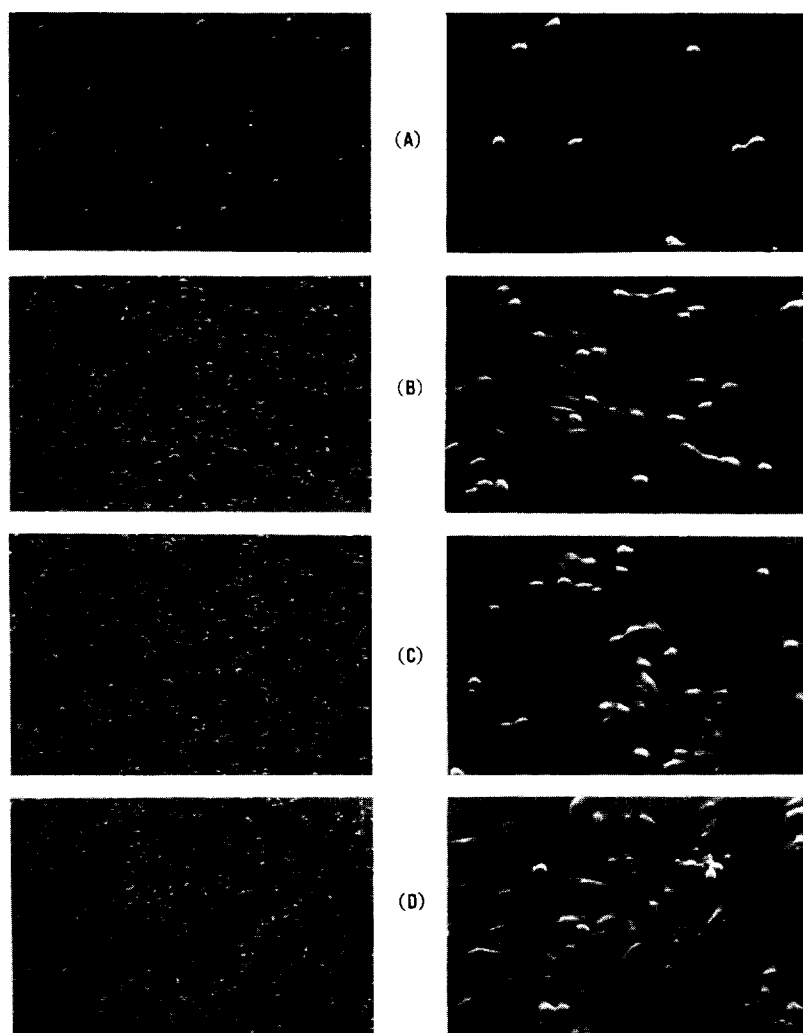


Fig. 6. Morphologies of CHO cells adhered and spread on PS dish surfaces at different magnifications (culture time, 2 hr ; medium with serum, 5% FCS ; SEM). (A) untreated, (B) tissue culture grade, (C) O₂ plasma-treated and (D) H₂O plasma-treated PS dish surfaces.

morphology of the CHO cells adhered and spread on the differently treated PS dishes. The cells were well distributed and attached to the surface-treated dishes after 2 hr culture (Fig. 6, $\times 100$). The water vapor plasma-treated dish surface showed better cell spreading than the tissue culture grade or oxygen plasma-treated one (Fig. 6, $\times 400$). Cell spreading on polymer surfaces implies that the cells are more compatible with those surfaces. The untreated PS dish surface is hydrophobic and the cells are not compatible with the hydrophobic surface,¹⁴⁻¹⁶ resulting that the cells are not well spread and have round shapes on the untreated surface. The surface-treated PS dishes used in this study have similar hydrophilicity and oxygen contents as discussed earlier. The main differences of the dishes are the functional groups produced on the surfaces; hydroxyl groups for the water vapor plasma-treated dish surface and hydroxyl, ketone and carboxyl groups for the tissue culture grade or oxygen plasma-treated one. It was reported in a previous paper⁵ that although wettability is an important factor for cell adhesion and spreading onto polymer surfaces, hydroxyl groups may be a more important factor. This is probably due to the specific hydrogen bondings between the surface hydroxyl groups of the polymer and the polar groups of the cell surfaces.¹

The results of this study convinced us that the surface hydroxyl groups positively affect the adhesion and spreading of cells on polymer substrates. The water vapor plasma treatment is a simple and effective method to produce hydroxyl groups on the polymer substrates. It is even simpler than conventional oxygen plasma treatment because a water container is used in this treatment instead of an oxygen gas tank. The PS dishes or flasks may be effectively treated for enhanced cell culture by the water vapor plasma treatment.

We have prepared hydroxyl group gradient polymer surfaces, where the hydroxyl group density changes on one polymer surface in a gradient along the sample distance.⁸ We expect that the cell

culture on those surfaces can give us clearer information about the effect of the hydroxyl groups on cell adhesiveness.

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