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Cell Culture on Polymers Prepared by Radiation-Induced Polymerization of Various Glass-Forming Monomers

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Abstract

The growth of cells on polymers prepared by the radiation polymerization of monomethacrylate and dimethacrylate was investigated. Cell growth was affected greatly by such properties of the polymers as water content, wettability, and porosity. Growth was promoted remarkably by rinsing the polymers with warm water at 60–70°C and by irradiation of polymers with an electron beam. Cell growth decreased with increasing oxyethylene length (n) in the polymerized dimethacrylate of same series, $CH_2C(CH_3)CO(OCH_2CH_2)_nOCOC(CH_3)CH_2$. A decrease in the hydrophilicity of the polymer increased cell growth rate. Formation of pore structures in the polymer films also increased the cell growth.

Index Entries: Cell culture, on radiation-induced polymers; radiation polymerization, and cell culture; polymers, cell culture on; glass-forming monomer, cell culture on; water content of radiation-induced polymers; wettability, of radiation-induced polymers; porosity, of radiation-induced polymers.

Introduction

Recently, synthetic polymers have been widely used for various biomedical purposes, and also as substrates in cell culture. Various materials, such as polymers coated with protein or polymers having increased hydrophilicity, hydrophobicity, and electron charges, were applied to the acceleration of cell growth (*1–5*). Poly-

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meric dishes for cell culture have been developed. Their effects on cell culture were determined by the kind of polymer and nature of the surface (*6, 7*). Systematic studies on the effects of various physical and chemical properties of polymer surfaces, such as porosity, electron charge, and hydrophilicity of cell culture have not been widely carried out. The effect of new polymer surfaces on the cell culture is also a problem of increased interest.

Many investigators have studied the immobilization of bioactive components such as enzymes (*8*), microbial cells (*9*), drugs (*10*), and tissue cells (*11, 12*) by means of radiation-induced polymerization (polymerization by γ-ray irradiation from ^{60}Co) of glass-forming monomers that have stable supercooling properties and ready polymerizability at the low temperature (*13, 14*). Even very unstable cells could be effectively and stably immobilized by radiation-induced polymerization of a suitable carrier. Radiation polymerized polymers do not contain a catalysis residue, and should therefore be advantageous for cell culture. Moreover, radiation polymerization is convenient for the preparation of porous polymer films.

In this report, the effect of properties and morphological structures of polymers on cell growth using radiation polymerized polymer were investigated.

Materials and Methods

Various polymer films were prepared to a thickness of 100 μm by casting during radiation polymerization. The monomers used were commercially available ethyleneglycol dimethacrylate (2G), triethyleneglycol dimethacrylate (3G), tetraethyleneglycol dimethacrylate (4G), polyethyleneglycol dimethacrylate (9G), neopentylglycol dimethacrylate (NPG), dipropyleneglycol dimethacrylate (P-2G), polypropyleneglycol dimethacrylate (P-9G), methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), glycidyl methacrylate (GMA), trimethylolpropane trimethacrylate (TMPT), and tetramethylolethane tetramethacrylate (TMET).

The polyvinyl fluoride (PVF) film was commercial grade (Du Pont Far East Inc., USA) with a thickness of 25 μm.

Glial cells (C_6) and pituitary tumor cells (GH_3) of rat origin and Chang liver cell-in-cell line growing as a monolayer in cell culture were used. The cells were layered on various polymer films and incubated in Eagle's Minimum Essential Medium (MEM) including 10% fetal calf serum (FCS) under an atmosphere of 5% CO_2–95% air at 37°C. The medium was exchanged after a first incubation of 1 d, then at intervals of 2 d. After cultivation, the cells adhering to the polymer were harvested using 0.25% trypsin in Ca^{2+}- and Mg^{2+}-free phosphate buffer at pH 7.4 for 15 min. They were collected by centrifugation at 800–1000 rpm and were calculated on blood account plates. The cells were observed during culture by CK-type phase contract microscopy using an Olympus Microscope (Tokyo). Cells adhering to the polymer during culture were fixed with 2.5% glutaraldehyde in phosphate buffer at pH 7.4 for 15 min and were then coated with a gold–palladium alloy. The morphology of the cells growing on the polymer was observed by scanning electron microscopy (Nippon Electro Co., Ltd.).

The water content of the polymer was calculated according to Eq. (1):

$$\text{Water content } (\%) = (Ws - Wp)/(Wp \times 100) \quad (1)$$

where Wp is the weight after drying and Ws is the weight after immersion in pure water for 2 d.

The irradiation of formed polymer was carried out using an electron accelerator under air at 25°C. The contact angle was estimated by determining the diameter and height of a waterdrop on the various polymers using a Kyowa Contact Angle Meter.

Results and Discussion

Effect of Wettability on Cell Growth

Previously, polymer substrates on cell cultures were often treated chemically or physically by any means to improve their surface for cell growth (*1–5*). Since the polymer films prepared by radiation polymerization of glass-forming monomers were transparent, cell growth on the polymers could be clearly observed by phase contract microscopy. Figure 1 shows growth curves of C_6 cells on triethyleneglycol dimethacrylate (3G) polymer treated by various methods. According to these results, the number of cells increased with increasing incubation time and in the later stages cell growth was retarded owing to confluency. The polymer surfaces were covered by spread cells and cell growth was retarded by contact with neighboring cells. The treated film showed faster growth of cells than that observed on the untreated film. Cell growth occurred most quickly on the polymer previously treated with warm water. The growth of cells on the film irradiated by the electron beam was almost same as that on a commercial falcon dish and on a glass plate.

The effects of irradiation of the 3G polymer and PVF films on cell growth are shown in Figs. 2 and 3. The irradiated 3G polymer and PVF films were more effective for cell growth. A suitable degree of wetting of the polymer substance with water is an important factor for the attachment and growth of cells. The wettability of the substrate can be estimated by measuring its contact angle. As seen in Figs. 2 and 3, the contact angle decreased and the wettability of the polymer increased with increasing irradiation dose. Cell growth on the polymer film was activated with decreasing contact angle. The glass surface has a smaller (20°) contact angle and also was wetted with water more easily than was the polymer. Therefore, cells could adhere and grew more easily on the glass surface. Further, as shown in Fig. 1, cell growth was increased on the 3G polymer surface by such treatments as rinsing with warm water. In this case, the contact angle of the rinsed 3G polymer film decreased from 52° in untreated polymer to 44°, showing that wettability is increased by such treatment. As shown in Figs. 2 and 3, cell growth increased on the irradiated 3G polymer and PVF. In these cases, the contact angle of the polymer increased with the irradiation. Therefore, it was concluded that wettability, expressed by the observed contact angle, is the most important factor affecting cell growth.

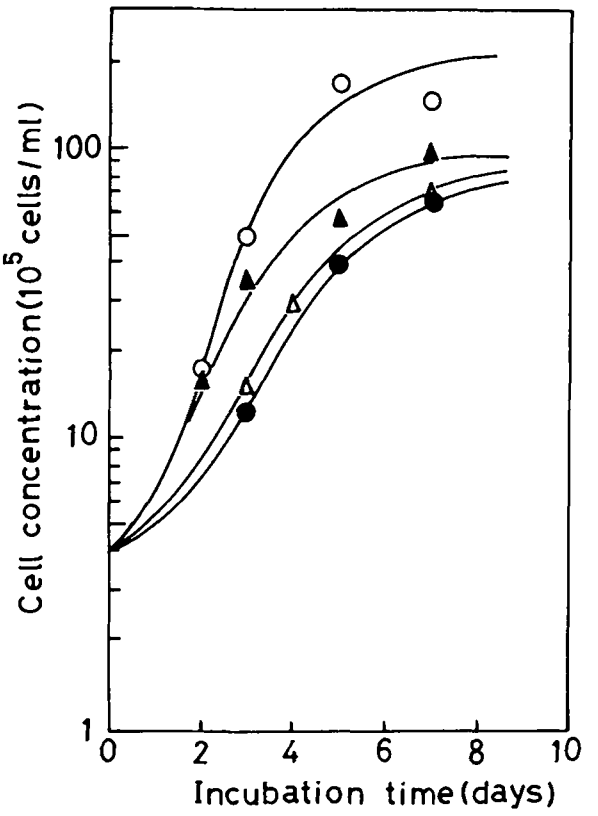

Fig. 1. Growth curves of C_6 cells on triethyleneglycol dimethacrylate polymer (3G polymer) by various treatments: (○) immersed in warm water for 5 h at 60°C; (△) sparked by tesla coil (tesla transformer) for 10 min; (▲) irradiated by electron beam generator, 20 Mrad; (●) untreated.

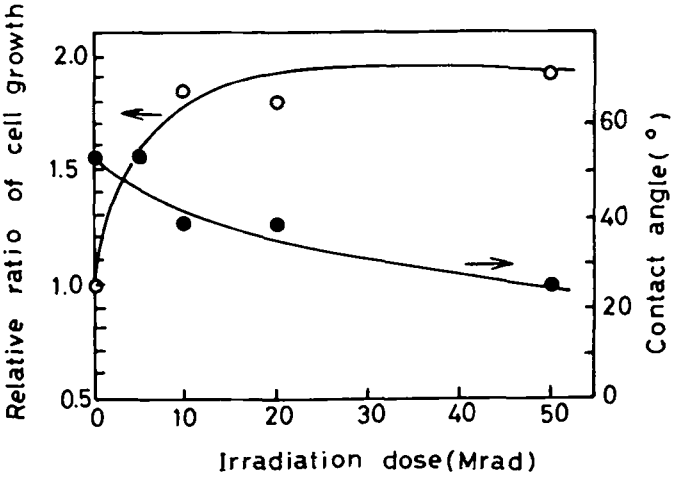

Fig. 2. Effect of irradiation of the 3G polymer on C_6 cell growth. Culture time, 3 d.

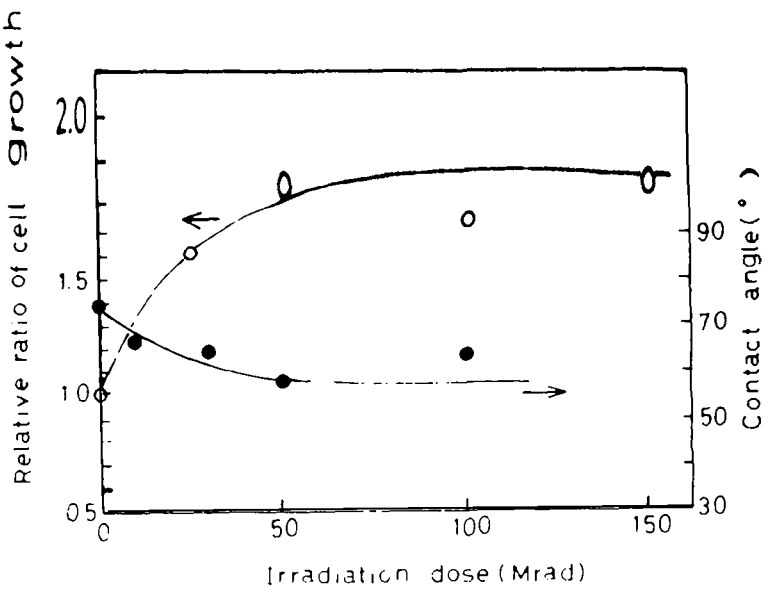

Fig. 3. Effect of irradiation of the PVF on C_6 cell growth. Culture time, 3 d.

Effect of Hydrophilicity on Cell Growth

Table 1 shows the change of the water content with the change in length of the oxyethylene unit (n) in polyethyleneglycol dimethracrylate polymer, $CH_2C(CH_3)CO(OCH_2CH_2)OCOC(CH_3)CH_2$. The water content of the polymer was changed by the length of the oxyethylene unit (n) and the hydrophilic property increased with the number of (n). Figure 4 shows the number of cells after 5 d incubation on the dimethacrylate polymer (n = 2–9). Cell growth decreased with increasing water content, that is, the length of the oxyethylene unit. The growth of GH_3 cell showed the similar tendency with that of C_6 cell. The growth of GH_3 cell on the polymer film was slower than that of C_6 cells, but the number of the GH_3 cells increased to about five times the original number immediately after adhesion on hydrophobic polymers such as 2G and 3G.

Cell culture on the other polymers were also investigated. Figure 5 shows the number of C_6 cells after 5 d of incubation. The cell number varied depending on the kind of polymers employed. The contact angles in these polymers were similar to each other and all fell in the range of 50–55°. The water contents of the various polymers were quite different. On those polymers having low water contents, such as 2G, 3G, P-2G, and P-9G, cell numbers increased greatly. On polymers with

TABLE 1
Change of Water Content by Length of Oxyethylene Unit (n) in Polyethyleneglycol Diemthacrylate Polymers

Monomer formula	n = G	Water content, %
$CH_2C(CH_3)CO(OCH_2CH_2)_nOCOC(CH_3)CH_2$	2	2.5
	3	4.5
	4	10.0

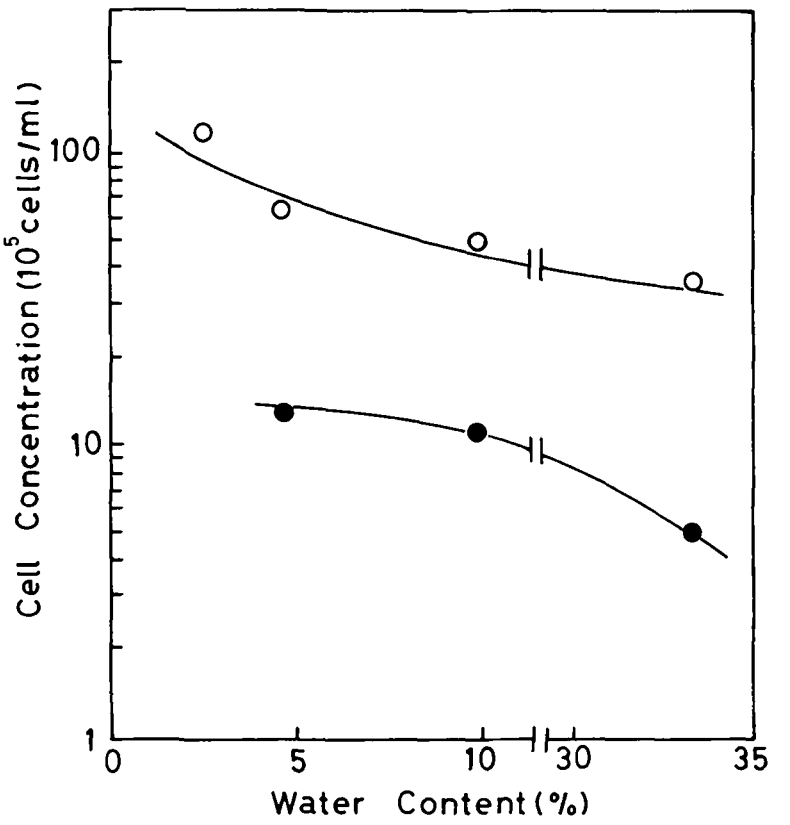

Fig. 4. Growth of C_6 and GH_3 cells on different oxyethylene chain length (n = 2–9) of polyethyleneglycol dimethacrylate polymer, $CH_2C(CH_3)CO(OCH_2CH_2)$-$OCOC(CH_3)CH_2$: (○) C_6 cell, (● GH_3 cell. Culture time, 5 d; implanted cells, 3×10^5 cells/mL.

higher water contents, such as HEMA and the 9G polymer, cell growth was remarkably retarded. Importantly, water content affected both cell growth on and the wettability of the polymer.

It is concluded that high wettability (contact angle) and low hydrophilicity (water content) are the preferred conditions for cell growth.

Effect of Porosity on Cell Growth

The degree of cell growth might be affected by other factors such as the smoothness of the polymer surface and the polarity of the polymers (*1–5*). The effects of microporous surface structure on cell growth were investigated. Such microporous polymers were prepared by radiation polymerization of monomers that included polyethylene glycol and ethylene glycol as pore making agents. These pore making agents were removed with water after polymerization to make the pore structure (*15*). Figure 6 shows cell growth on porous polymer films prepared by polymerization at low temperature, −78°C. Cell growth was promoted slightly by increasing

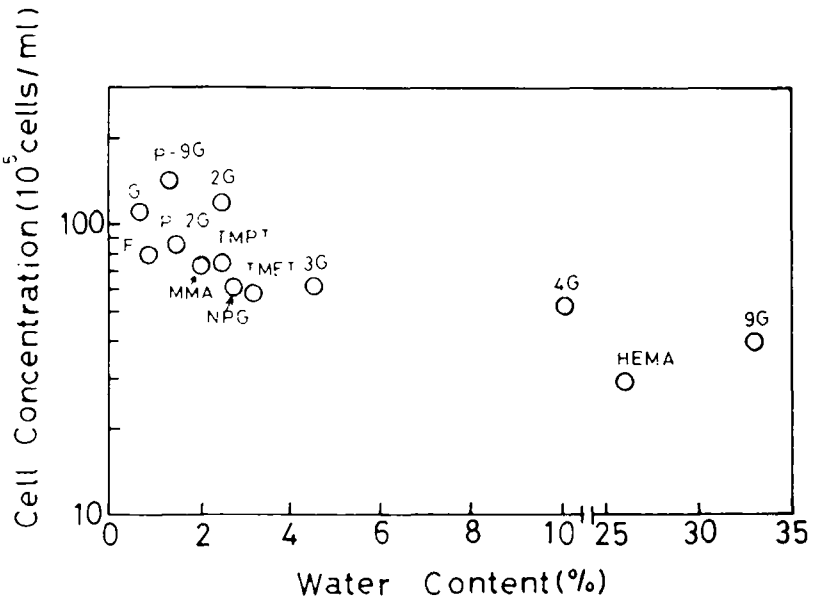

Fig. 5. Culture of C_6 cells on various polymers. Culture time, 5 d: implanted cells, 3×10^5 cells/mL; NPG, neopentylglycol dimethacrylate; P-2G, dipropyleneglycol dimethacrylate; P-9G, polypropyleneglycol dimethacrylate; TMPT, trimethylolpropane trimethacrylate; TMET, tetramethylolethane tetramethacrylate; MMA, methyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; G, glass; F, falcon dish; 2G, diethyleneglycol dimethacrylate; 3G, triethyleneglycol dimethacrylate; 4G, tetraethyleneglycol dimethacrylate; 9G, polyethyleneglycol dimethacrylate.

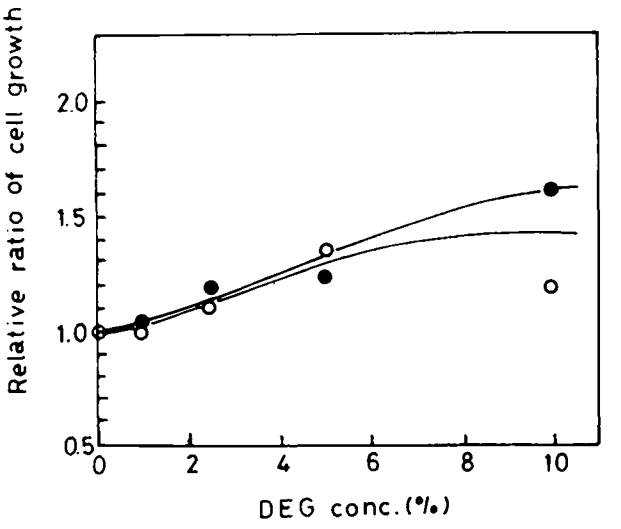

Fig. 6. Culture of liver and C_6 cells on polymer prepared by polymerization at −78°C: (○) C_6 cell; (●) liver cell. Culture time, 3 d.

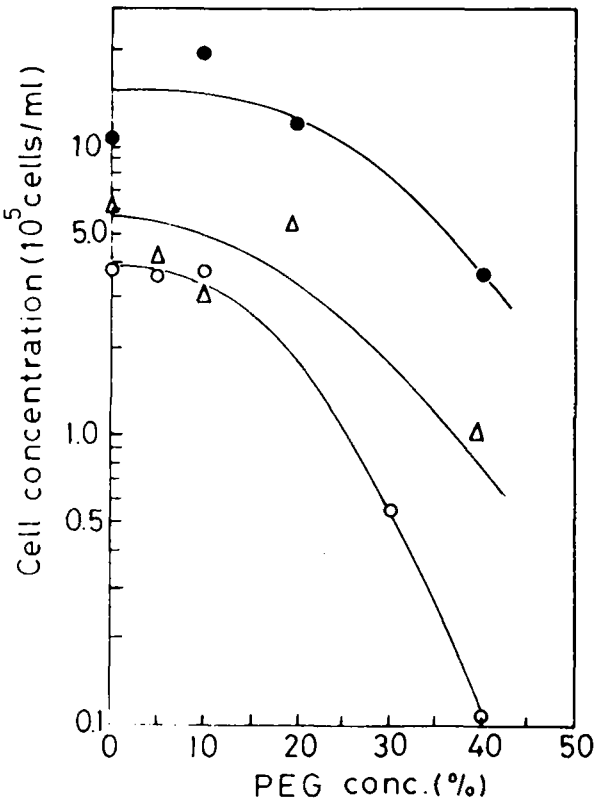

Fig. 7. Cultures of liver, C_6, and GH_3 cells on a polymer prepared by polymerization at 25°C: (○) C_6 cells; culture time, 3 d, implant cells, 0.63×10^5 cells/mL; (△) liver cells; culture time, 3 d; implant cells, 0.56×10^5 cells/mL; (●) GH_3 cells; culture time, 5 d; implant cells, 1.3×10^5 cells/mL.

the content of diethyleneglycol, that is, with increasing porosity. Figure 7 shows cell growth on porous films prepared by polymerization at room temperature, 25°C. Cell growth on the polymers was decreased sharply with increasing PEG concentration. The difference between the two results is caused by the difference in their porous structures. Figure 8 shows the porous structure of the films prepared at −78°C, observed by means of phase contact microscopy. In this case, few pores were found on the polymer surface. But many pores were observed inside the film and below the surface. This inner porosity might promote the transmittancy of oxygen and nutrition through the membrane and produce a positive effect on cell growth, as shown in Fig. 6. On the other hand, cell growth decreased on the porous polymers (Fig. 7) formed at 25°C. Figure 9 shows the porous structure of films prepared at 25°C. In this case pore structure could be clearly observed on the polymer surfaces. Therefore, it was concluded that the surface pore structure had a negative effect on cell growth, though the porosity inside the polymer film had a positive effect on cell growth.

It was found that various physical and chemical properties of polymeric materials, such as water content, wettability, and pore structure, were important factors

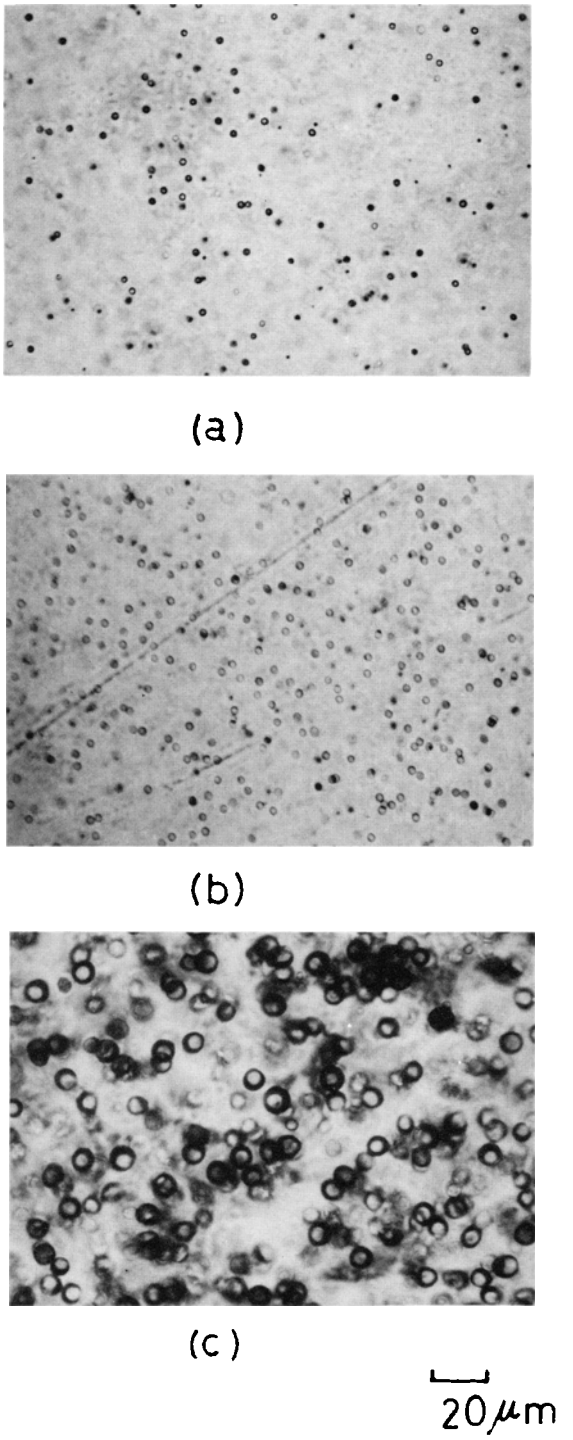

Fig. 8. Phase contrast microphotographs of the porous polymer films formed by rinsing the diethylene glycol in the polymer with water after polymerization at −78°C. Diethylene glycol concentration: (a) 1%; (b) 5%; (c) 10%.

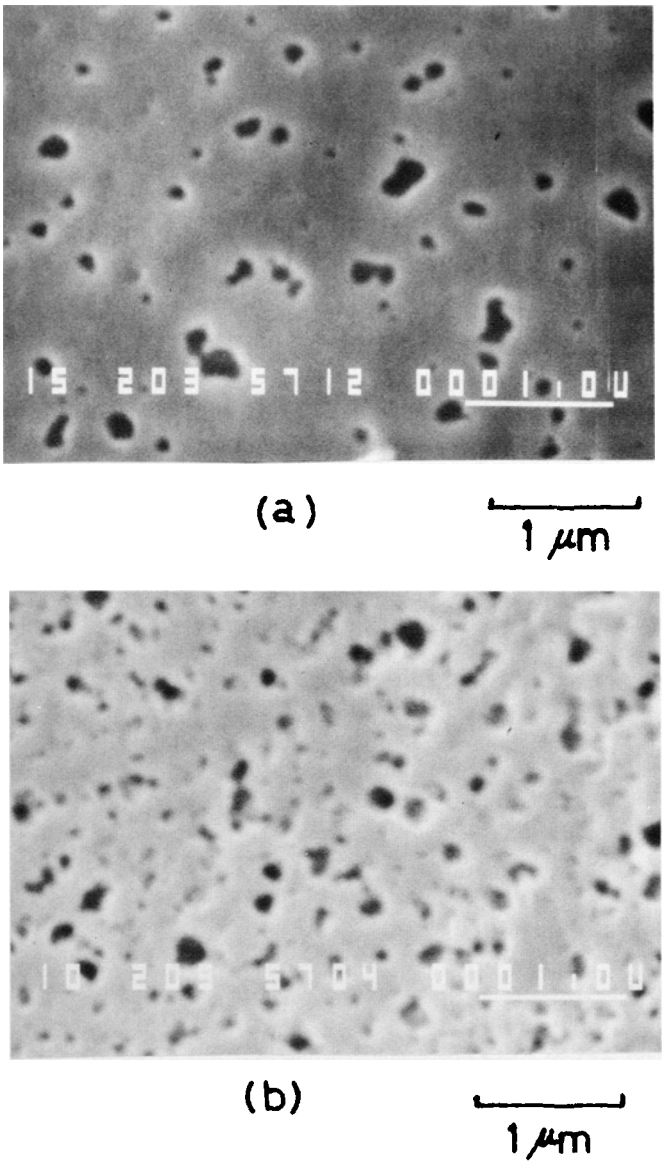

Fig. 9. Scanning electron microphotographs of the porous polymer films formed by rinsing the polyethylene glycol in the polymer with water after polymerization at 25°C. Polyethylene glycol concentration: (a) 20%; (b) 40%.

for cell culture. These results should give fundamental information for the design and selection of polymer substrates and also techniques for the control and promotion of cell growth that will be useful in developing and applying cell culture processes to the production of hormones, enzymes, antibodies, and other physiologically or biochemicaly active substances.

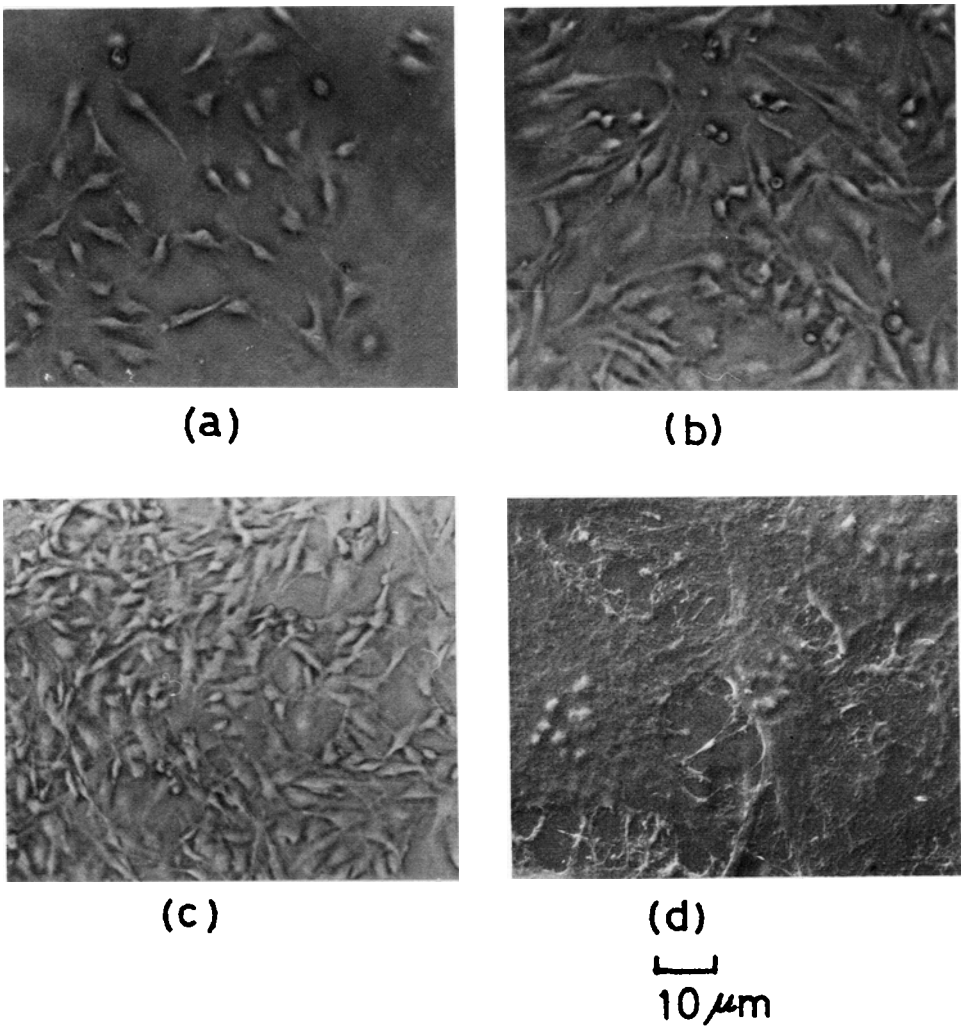

Fig. 10. Phase contact microphotographs (a–c) and scanning electron microphotographs (d) of C_6 cells growing on 3G polymer film. Culture time: (a) 1 d; (b) 2 d; (c and d) 5 d.

Observation of Cell Growth

When cells adhere to the substrate surface, they begin to grow by spreading across the substrate (*16*). Figure 10 shows phase contract microphotographs and scanning electron microphotographs for various stages of C_6 cell growth on 3G polymer. According to these photographs, the number of adhered cells on the polymer increased with incubation time by cell division. The polymer surface was almost covered by cells after 5 d. These observations correspond well to the culture curve shown in Fig. 1. Furthermore, the cells were spread and adhered flatly and widely on 3G polymer (photograph, 10d). The rate of cell growth on higher hydrophilic polymers such as 9G and HEMA was slower than that on higher hydrophobic poly-

mers such as 2G and 3 G, and the morphology of adhesion and growth were hardly changed between the hydrophilic and hydrophobic polymers.

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