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Cell Culture on Polymers Prepared by Radiation-Induced Grafting of Various Monomers

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Abstract

The adhesion and growth of tissue cells on polymers prepared by radiation grafting was investigated. The apparent rates of initial attachment and growth of Chang liver and C_6 cells were promoted on surfaces with increased wettability and with a heterogeneous structure for grafted polyvinyl fluoride film. The degree of cell attachment and growth on surfaces having a dense microblock structure, formed by grafting of methyl methacrylate in acetone solvent, was greater than that caused by other factors, such as wettability.

Index Entries: Cell culture, on grafted polymers; radiation-induced polymers, cell culture on; wettability of polymer; monomer; flat polymer surface, cell culture on; polymer surface, cell culture on nonflat; polymer film, cell culture on.

Introduction

Recently, cell culture has been studied actively by various workers for the production of useful biological substances (*1–3*) such as hormones, enzymes, and antibodies in in-vitro systems and also for the development of the hybrid artificial organs (*4, 5*), such as artificial livers and pancreases. For this purpose, the study

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of substrate materials to promote and control the adhesion and growth of cells becomes a very important problem since the rate of cell growth might be greatly affected by the hydrophilicity, surface smoothness, and electronic charge of a substrate (*6–9*). The effects of some treatments of polymers, such as the use of irradiation or plasmas to modify the surface properties of polymers to promote cell growth, were investigated. Furthermore, the effects of increasing the affinity between the cells and polymer by coating them with specific biosubstances, such as fibrins, collagens, and proteins, were also reported (*10,11*).

In a previous paper (*12*), it was shown that lower water content and the porosity were relatively effective in increasing cell growth owing to the increased wettability of polymer surfaces. It is well known in the field of radiation chemistry that graft polymerization changes the surface properties of polymers and thus might cause a desirable change that would result in improved cell culture.

In this report, the effects of radiation-induced graft polymerization in modifying the wettability and smoothness of polymer surfaces in cell cultures was investigated.

Materials and Methods

The polyvinyl fluoride (PVF) films with thickness of 25 μm were used for grafting. The graft monomers used were 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) purified by distillation under reduced pressure.

The radiation grafting was carried out as follows. The mixture of monomer and solvent was introduced into glass ampules including 8 × 10 cm film. Then the samples were deaerated by bubbling nitrogen, and irradiation was carried out with γ-rays from a ^{60}Co source at 25°C. The degree of grafting was modified by changing the dose rate and irradiation time. After grafting, the graft polymer was washed, using acetone to remove homopolymer and residual monomers, and dried under reduced pressure at 25°C.

Glial cells (C_6) of rat origin and the Chang liver cell-in-cell line growing as a monolayer in cell culture were used. The cells were layered on various polymer films placed at the bottom of a Petri dish of 2.5 cm diameter and incubated in Eagle's Minimum Essential Medium (MEM), which included 10% fetal calf serum (FCS) under an atmosphere of 5% CO_2–95% air at 37°C. The medium was exchanged after the first day of incubation, and then at 2-d intervals. The number of initially attached and growing cells on the films were measured as follows: The cells adhering to the polymer were harvested by 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 count plates.

The cells adhering to the polymer during cultivation 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 contact angle was estimated by determining the diameter and height of the water drop layered on the various polymers using a Kyowa Contact Angle Meter.

Cell Culture on the Various Substrates

The culture of cells on various fluoropolymers were investigated. The results are shown in Table 1. The degree of cell growth differed by the kind of polymers. It was the greatest in the PVF film among fluorine-containing polymers. The cell growth rates were greater on the plates of smaller contact angle, such as glass and a commercial plastic dish. The reason can be attributed to the wettability of the substrate surface. That is, cell growth was affected largely by the wettability of the substrates. The contact angles of fluorine-containing polymers are very different from those of glass and the plastic dish. Figure 1 shows the culture curves of C_6 and liver cells during incubation on PVF film. The cell growth increased with culture time and the cell number reached about ten times the original value after 3 d. Moreover, the cell growth were retarded by contact inhibition after 6 d. The rate of cell growth was quite similar to that of both of C_6 and liver cells on PVF film.

Formation of Microheterogeneous Structure on Polymer Surfaces by Grafting

In the previous paper (*12*), it was found that formation of micropore inside polymer films were effective for cell growth. The effect of polymer surface heterogeneity produced by grafting onto initial cell attachment and cell growth were investigated. The polymer surfaces formed a heterogeneous structure easily by

TABLE 1
Cell Culture on Various Substrates[a]

Substrates	Contact angle (degree),°	Cells/mL	
		C_6	Liver
Polyvinyl fluoride (PVF)	78.3	9.6×10^5	12.7×10^5
Polyvinylidene fluoride (PVDF)	75.0	4.0×10^5	7.8×10^5
Polytetrafluoro-ethylene (PTFE)	80.7	7.2×10^5	—
Copolymer of tetrafluoroethylene and ethylene (Acron)	81.3	5.4×10^5	7.4×10^5
Copolymer of tetrafluoroethylene and perfluoro vinylether (PTA)	104	6.0×10^5	—
Copolymer of hexafluoropropylene and tetrafluoro-ethylene (FEP)	95.7	3.4×10^5	—
Glass	20.0	17.3×10^5	19.8×10^5
Falcon dish	28.0	22.6×10^5	18.3×10^5

[a]Implanted cells, 1×10^5 cells/mL; culture time; 3 d.

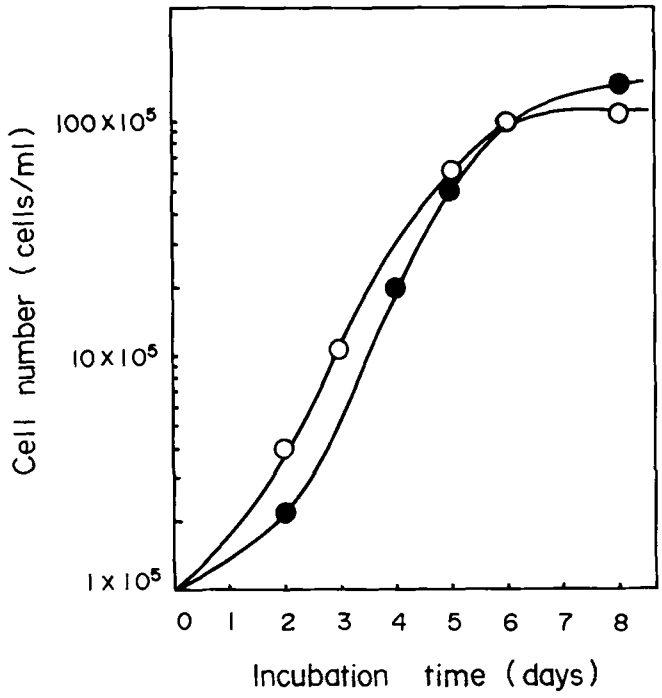

Fig. 1. Culture curves of liver and C_6 cells on PVF film: (○) liver, (●) C_6.

controlling grafting conditions such as type of monomer used and the solvents. The cells were cultivated on the different heterogeneous polymer films. Figure 2 shows microphotographs of polymer surfaces grafted with various monomers. The MMA monomer was grafted on PVF in solution of different solvent, such as *N,N*-dimethylformamide (Figs. 2b, c) and acetone (Figs. 2d, e). In case of MMA-grafted PVF, the degrees of microheterogeneity in the polymer surfaces were very different, depending on the solvents used in the experiment. In the film grafted in acetone, many microblocks of grafted MMA polymer were observed on polymer surfaces and the density of the microblock structure increased with increasing grafting ratio.

Initial Cell Attachment and Cell Growth on Film Endowed Microheterogeneity by Grafting

The structures of the polymer surfaces were important for cell attachment immediately after implanting and for centrifugel growth of filopodia during culture. As shown in Fig. 2, polymers of different surface structures were prepared. Figure 3 shows relative ratio of cell growth after 3 d in culture on grafted films with MMA in DMF and acetone solvents. In the case of cell cultures on MMA-grafted film in acetone, the C_6 and liver cell growths increased with increasing grafting ratio. On the other hand, cell growth was hardly changed by the grafting ratio of grafted films in DMF solvent. As seen in Fig. 2, this suggested that cell growth was af-

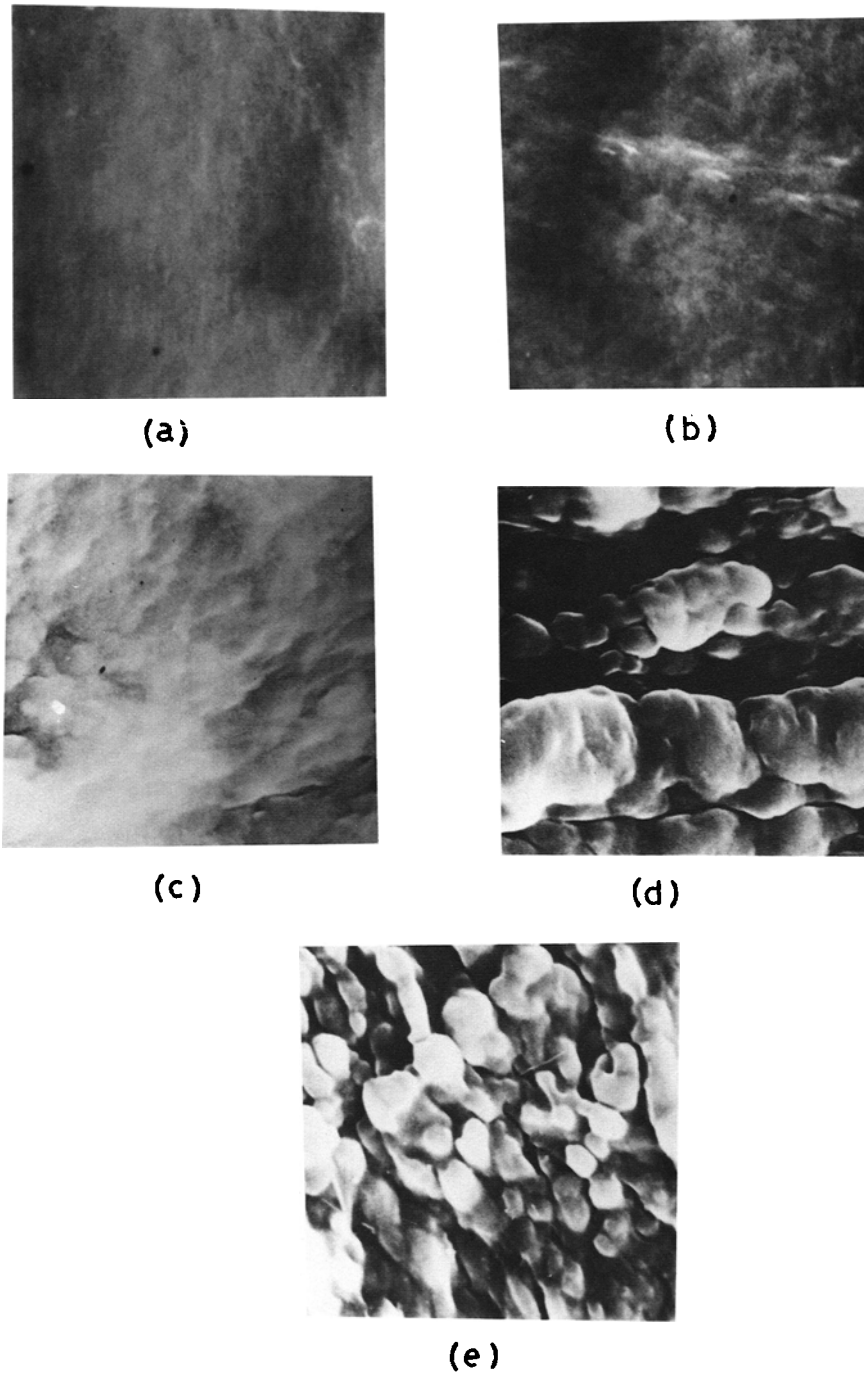

Fig. 2. Photographs of grafted polymer surfaces. (a) original PVF (before grafting); (b) grafting of MMA in DMF solvent, degree of grafting, 17.0%; (c) grafting of MMA in DMF solvent, degree of grafting, 50.6%, (d) grafting of MMA in acetone solvent: degree of grafting, 13.6%, (e) grafting of MMA in acetone solvent, degree of grafting, 34.3%

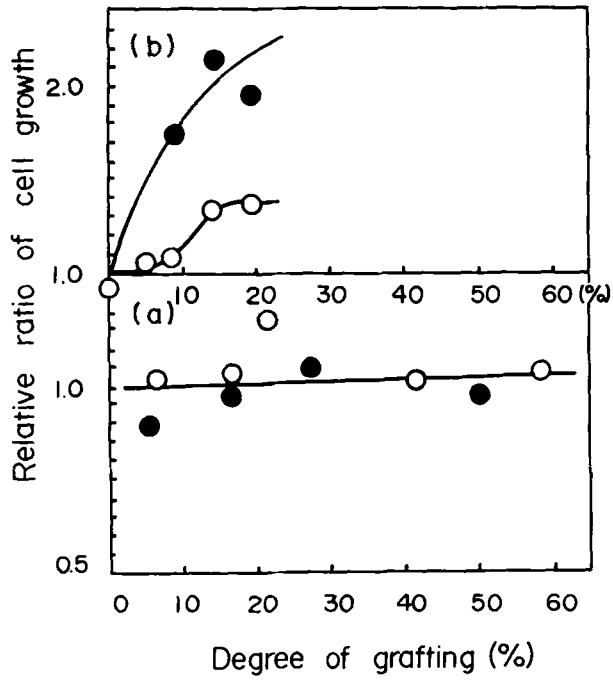

Fig. 3. Relative ratio of cell growth in incubation on PVF film-grafted MMA in DMF and acetone solvents: (○) liver cells, (●) C_6 cells, culture time, 3 d (*a*) grafted MMA in DMF, (*b*) grafted MMA in acetone

fected by the degree of heterogeneity in the grafted film. Furthermore, when cells were cultivated on various materials, the cells begin to spread after attachment on the polymer surfaces. The initial cell attachment after 5 h from implanting was investigated. The results are shown in Fig. 4. The initial cell adhesion indicated almost the same tendency with change of the cell growth after 3 d, as shown in Fig. 3. An increase in the initial level of cell attachment to the grafted polymer films promoted cell growth during culture after adhesion. It is sure that the polymer surface was formed by grafting the heterogeneous block structure on a microscale. The block has a size of approximately 1–3 μg. This size is smaller than the cell's size of 10–15 μm. As shown in Fig. 3, the degree of C_6 cell growth on the heterogeneous surface reached about twice the original cell growth on PVF film. This cell growth ratio was greater when compared to cultures grown on films of increased wettability produced by irradiation of an electron beam generator as described in the previous paper (*12*).

Cell cultures on poly(methyl methacrylate), segmented polyurethane, and segmented polyester films with three different types of surface roughness was reported by Y. Imai (*7*). On rough surfaces, initial cell attachment was promoted, but the rate of cell growth was inhibited compared with smooth surfaces. The results of cell growth differed from cultures grown heterogeneously by grafting, since the surface structure formed by Y. Imai is rougher than that produced by our method of 1–5 μm. Thus, it was deduced that differences in the microblock size formed on polymer surfaces affected cell growth. The degree of initial cell attach-

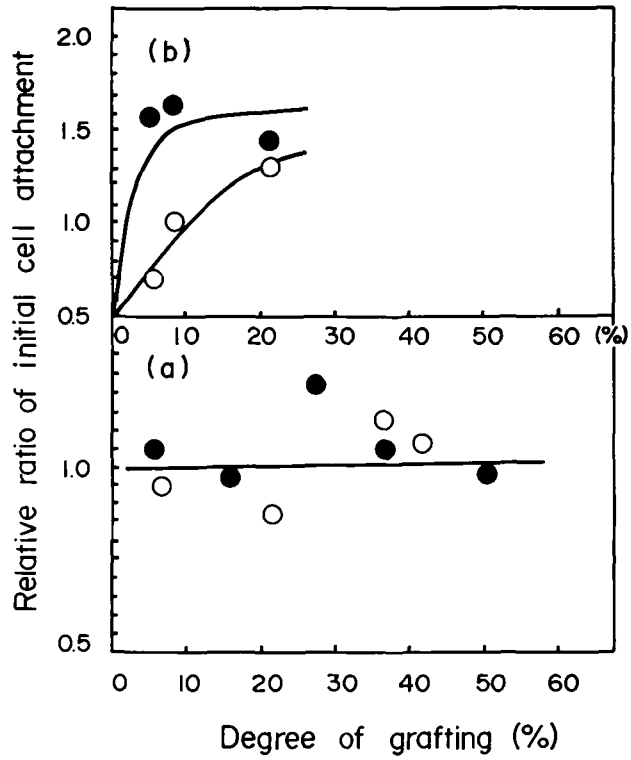

Fig. 4. Relative ratio of cell adhered in incubation for 5 h on PVF grafted MMA in DMF and acetone solvents: (○) liver cells, (●) C_6 cells, culture time, 3 d. (a) grafted MMA in DMF, (b) grafted in acetone.

ment and cell growth of the cells on heterogeneous films was almost the same as that on surfaces of plastic dishes and glass plates. It was concluded that surfaces having microblock structure were favorable for attachment and growth of cells.

Cell Culture on Substrates of Increased Wettability

The hydrophilic monomers, such as 2-hydroxyethyl methacrylate and methacrylic acid, were grafted on PVF film, endowing hydrophilicity. Figures 5 and 6 shows the cell growth on grafted PVF of increased wettability. The contact angle decreased and the wettability increased by grafting the hydrophilic monomers. The amount of cell growth increased greatly on the PVF film of lower grafting ratio, while it decreased on the PVF of higher grafting ratio. Especially, on methacrylic acid-grafted PVF, the extent of cell growth was changed greatly by the grafting ratio. The degree of cell growth on lower grafting ratio films was smaller in comparison by 1.8 times that of the original PVF film when cultured on films with increased wettability prepared by oxidation through irradiation with an electron beam generator, as described in the previous paper (*12*). Also, we reported that cell growth was remarkably retarded on polymers of high water content. In this case, although the contact angle of polymer was decreased by the grafting of hydrophilic monomers, the polymer surfaces were swelled by absorbing the me-

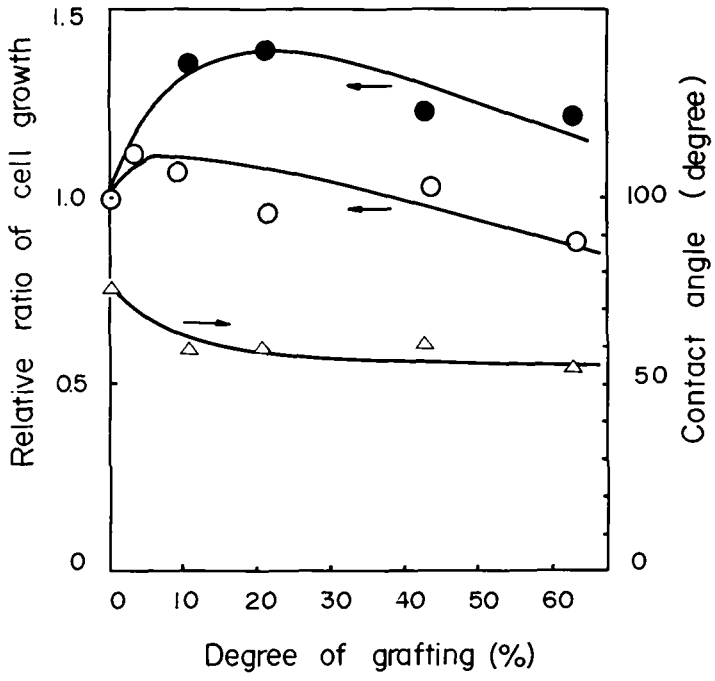

Fig. 5. Culture of liver and C_6 cells on PVF-grafted HEMA: (●) liver, (○) C_6, culture time, 3 d.

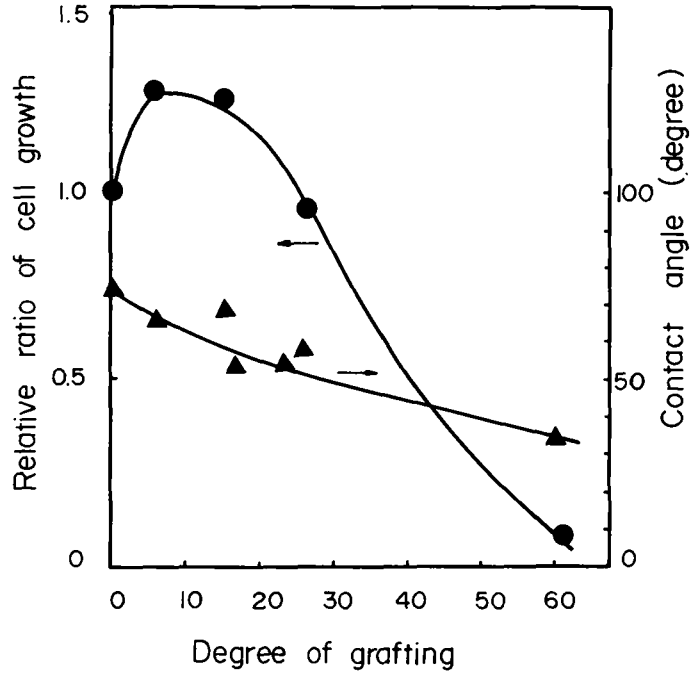

Fig. 6. Culture of liver cells on PVF-grafted methacrylic acid; culture time, 3 d.

dium. So, the culture rate on polymer-grafted hydrophilic monomers was smaller than that on irradiated films by electron beam generator.

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