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CELL GROWING WITH ACTIVATED CARBON FIVERS – SIGNIFICANCES OF POROUS SIZE –

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This report showed that which activated carbon fivers (ACFs) are favorable to contact and grow mammalian cells in vitro. ACFs used here were A-10, A-15 and A-20. In order to clarify a factor of different biological responses to ACFs, porous carbon films of poly(urethane-imide) have been investigate to determine an adequate porous size to cell survival in vitro. A cell-line used here was B16 murine melanoma cells. As a result, a better growing of cells was found in A-20 of ACFs and optimal porous size was around 1.2 μm.

Keywords: activated carbon fivers; poly(urethane-imide); porous size; cell growth

Previously, biological (pathological and immunological) responses to A-10, A-15 and A-20 of activated carbon fivers (ACFs) have reported from our laboratory. A-20 of ACFs was less inflammatory than others. It has been suggested that these differences between ACFs may be dependent on porous size of the surface of ACFs, however, it has not been investigated directly to use cells. Here, we tested where cells survive and grow with ACFs and whether an optimal porous size of a surface of materials is needed to survive or grow cells.

B16 murine melanoma cells used here were purchased from (American Type Culture Collection, USA). Cells were seeded at a density of 10^6/5 ml/ ϕ 6 cm dish in RPMI1640 medium (Nissui Pharmaceutical Co., Japan) containing 10% heat-inactivated FBS (ICN Biomedicals, Inc., USA) and induced to differentiate by treatment with 0.1 μM TPA (Sigma Chemical

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TABLE 1 Comparative Properties of ACFs

	A-10	A-15	A-20
Relative surface (m^2/g)	1,000	1,500	2,000
Pore volume (ml/g)	0.5	0.8	1.1
Ratio of pore vs surface (ul/m^2)	0.500	0.533	0.550

Co., USA) in a 5% CO_2 atmosphere at 37°C for 2 days. The cells were then washed in Dulbecco's phosphate buffered saline without calcium and magnesium (PBS) once and culture medium were changed to fresh RPMI1640 medium containing 10% fetal bovine serum (FBS). Cells were incubated together with sterilized materials such as ACFs or porous thin films, which were attached on the base of the incubation dish. A-10, A-15 and A-20 of ACFs were provided from Osaka Gas Co. LTD (Japan). Those physical properties are shown on Table 1 (data from Osaka Gas).

A porous thin film was made following the previous report by Takeichi *et al.* [1]. The brief method was that porous carbon films were prepared by the pyrolysis of poly(urethane-imide) films, which were prepared by a reaction between phenol-terminated polyurethane prepolymer and poly-(amide acid) obtained from pyromellitic dianhydride (PMDA) and 4,4′-oxydianiline (ODA) with a thermal treatment. A final porous size of films was 0.6, 1.2 and 3.0 μm on the average. After incubation for 24 hours, the culture medium was removed. After 5–10 min the plate was dipped several times in phosphate buffer solution (pH 7.4) to remove unattached biological materials. Then, each sample was prefixed in cold 2% glutaraldehyde in phosphate buffer solution for one hour for an observation by a scanning electron microscopy (SEM). Each sample was subsequently dehydrated with graded ethanol solutions, and dried with carbon dioxide by the critical point method. The dried surfaces were mounted and sputter-coated with goldpalladium and carbon. The samples were sputter-coated with 50 Å of gold-palladium and observed at 15 keV in a JEOL-JSM6000F scanning electron microscope.

In an incubation of cells with A-10 or A-15 of ACFs, almost cells did not adhere on the base of the dish and died. Figure 1 shows an aspect of the A-10 or A-15 of ACFs after the incubation. The growing aspect of cells such as adhesion and extension did not find any fields under the microscope.

Contrastingly, well growing of cells were found in the incubation with A-20 of ACFs as shown in Figure 2. Cells adhered on the fivers, and the surface of fivers was covered smoothly with the cell. A cell was communicated to another cells by an extension of a cellular surface, indicating that cells well grown and survived with the material of A-20 of ACFs. However,

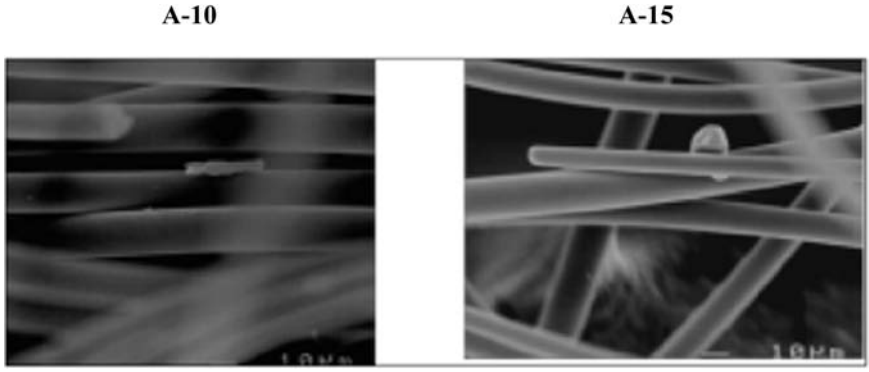

FIGURE 1 Figures show SEM aspects of cell culture with A-10 and A-15 of ACFs.

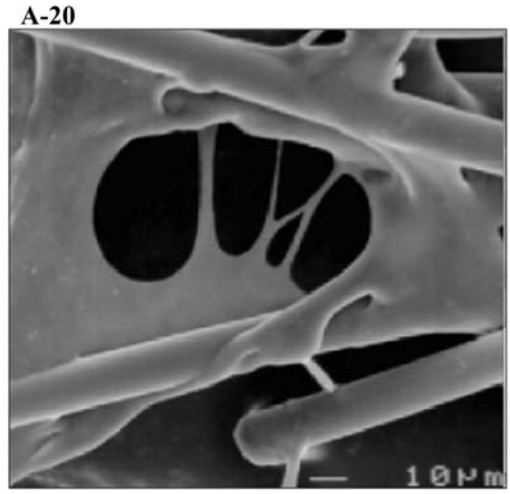

FIGURE 2 Figure shows a SEM aspect of cell culture with A-20 of ACFs.

these differences are not distinguished from a data of physical classification of ACFs shown in Table 1. A pore size of ACFs was hypothesized as a major candidate to cause such biological alterations, resulting from our accumulating observations of samples. To clarify these cellular affinities to ACFs, thus, we designed porous carbon film with several diameter of pore size. Investigating of porous thin films, then, we found no evidence of cellular survival on the films with pore size of 0.6 and 3 μm. All of cells on these films died during the incubation of 24 hours, investigating of cell fragments alone on the film under all fields of samples.

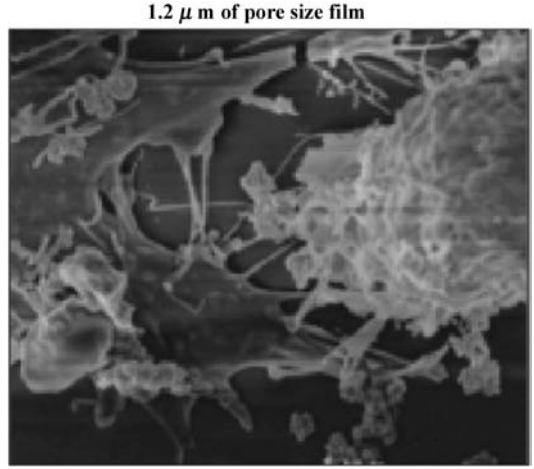

FIGURE 3 Figure shows cellular growing on the film with a average pore size of 1.2 μm.

However, as shown in Figure 3, cells based on the film with the porous size of 1.2 μm showed a better survival and growing, indicating that better incubation with a material such as a carbon film demonstrated here is needed an optimal pore size of the surface. The optimal size is around 1.2 μm at a range between greater than 0.6 μm and smaller than 3.0 μm. It has been shown that an optimal porous size was necessary to be survived and grown mammalian cells for their base, identically to the report of Dalton *et al.* [2]. This biological mechanism of a selection of pore size on cells has not been clear at the present time, however, several possibilities arise. Those factors may be related to several biological signal transudations such as diffusion gradient of growth factors, cell-cell communication and an optimal electron charge distribution [3]. To clarify those possibilities is lead to further biological significances. In this paper, we provide an importance of pore size of carbon composites materials for those biological applications.

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