Heat Shock Protein 70B mRNA Expression in L929 Cells Attached on Lipid Films

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(Received August 26, 1999; CL-990738)

L929 cells adhesion and function on the lipid films prepared by casting method were studied. The function of the cells adhered on to lipid films was estimated by HSP70B mRNA analysis. It comes clear that L929 cells adhered on the synthetic lipid films suffered some stress from the substrates.

Lipid films have many advantages as the basic substrates for studying cell-material interactions. It is easy to prepare the desired surfaces with various characteristics using adequate lipids.[1-3] The interaction of lipid films with bio-colloid were studied from the points of view of blood coagulation cascade and its regulatory pathways,[4,5] while the interaction between lipid films and cells was not fully understood yet.[6] In order to develop a novel bio-functional material, it is essential to know the interaction between cells and materials in detail. In previous studies,[7-9] we introduced a novel research strategy, the evaluation of mRNA expressions of the cells that came into contact with polymer surfaces using reverse transcription-polymerase chain reaction (RT-PCR) method. With that method, one can estimate the response of cells to polymeric materials at high sensitivity. In this study, the interaction between cells and lipid films was estimated by mRNA expression to obtain the basic knowledge of cell-material interaction. As a preliminary study, lipid films of simple composition were prepared.

The lipids used in this study were as follow: L-α-dipalmitoylphosphatidylcholine (DPPC), L-α-dipalmitoylphosphatidylethanolamine (DPPE), L-α-dipalmitoylphosphatidylserine (DPPS) D-α,β-dioctadecyl-glycerol ($2C_{18}$-Gly-OH), N-(α-trimethylammonioacetyl)-dioctadecyl-D-glutamate chloride ($2C_{18}$-D-Glu-C_2N^+), and sodium 1,2-bis(octadecyloxycarbonyl)-ethane-1-sulfonate ($2C_{18}$-SUC-SO_3^- Na^+). The lipid films were prepared by casting method onto Cell Disk (Sumitomo Bakelite, Tokyo, Japan). After cast, samples were washed by saline for 3 times and set in 24-multi well. Mouse fibroblasts (L929 cells) were cultured on lipid films using fetal calf serum (FCS) free and FCS added Eagle MEM, respectively. The mRNA we focused on was that of heat shock protein 70B (HSP70B). HSP70B is known as a member of heat shock proteins and one of the most sensitive markers for studying weak signals, such as physical or physicochemical stimulation that is caused by cell-material interaction.

Figure 1 shows the photographs of cells attached on the various surfaces of the films after 24 h incubation period at 37 °C. It was clear that L929 cells adhered on all lipids films as well as Cell Disk. Many researchers reported that initial cell adhesion onto solid surfaces was explained by the work of adhesion theory.[10,11] Based on the theory, the L929 cell number adhered onto some lipid films (DPPC and $2C_{18}$-D-Glu-C_2N) should drastically be reduced, because the water contact angles (suggested in Figure 1) of those lipids films were very low. In addition, static electricity also did not affect the cell adhesion

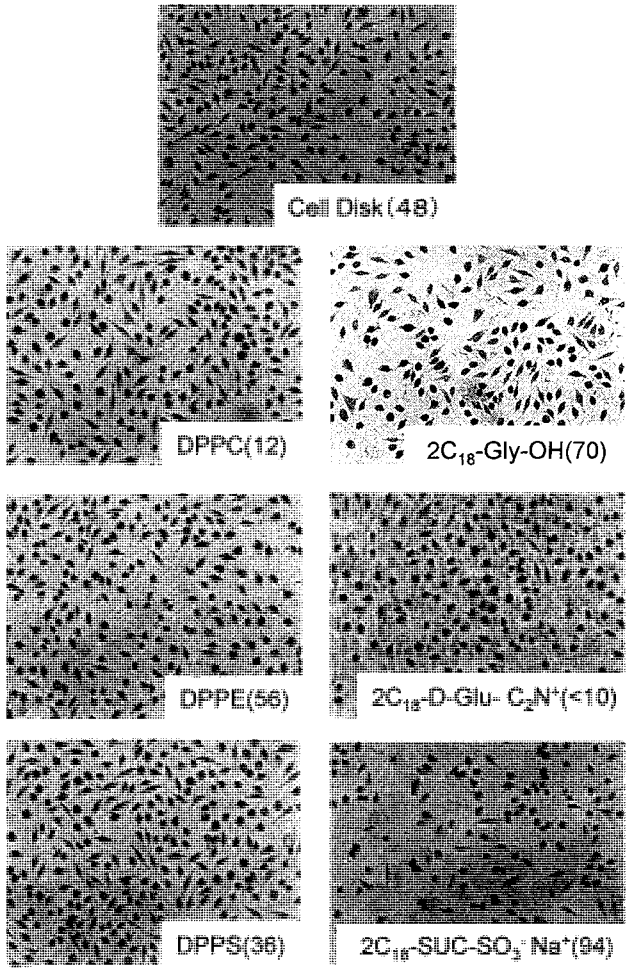

Figure 1. Optical micrographs of L929 cells adhered on various substrates after 24 h incubation. Each number means the water contact angles of the surfaces.

onto lipid films. The charged surfaces are known to interact with proteins and cells, while there were no significant differences among all the samples. These results show that the cell adhesion onto lipids films was different from that onto solid surfaces and that lipid film has some special interaction with proteins or cells.

The expression of HSP70B mRNA was determined by RT-PCR analysis.[12] Figure 2 shows the results of HSP70B mRNA expression in L929 cells adhered to various surfaces. When L929 cells were cultured using FCS free medium, HSP70B was expressed in the cells on all the samples. On the other hand, when the cells culture using the medium which was added 10% FCS, HSP70B expression depended on the samples

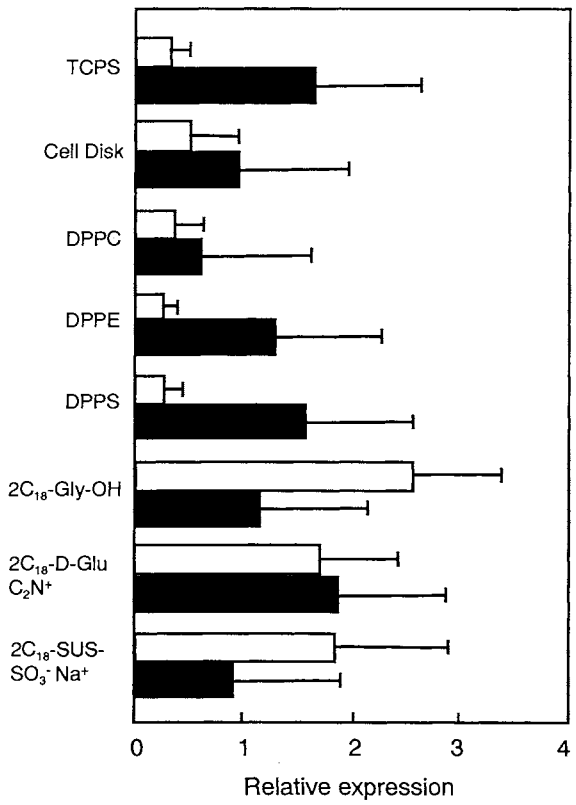

Figure 2. HSP70B mRNA expression in L929 cells adhered onto various substrates after 24 h incubation. Open columns means the data obtained using FCS added Eagle MEM, and closed columns means that of FCS free one, respectively. The relative values were expressed in logarithmic scale at arbitrary unit. All the data are triplicate and averaged.

that the cells adhered. On DPPC, DPPS and DPPE films, HSP70B mRNA expression in L929 cells were low as well as tissue culture polystyrene (TCPS), whereas on the rest three lipids films, the HSP70B mRNA levels were fairly high. It means that the cells adhered on the $2C_{18}$-Gly-OH, $2C_{18}$-D-Glu-C_2N^+, and $2C_{18}$-SUC-SO_3^- Na^+ films were suffered some stresses.

In previous study, we have shown that the cells adhered or attached on hydrophilic surface expressed high level of HSP70B mRNA.[9] It is known that protein adsorption and cell adhesion on hydrophilic surfaces were low, therefore, the high HSP70B mRNA expression on the hydrophilic surfaces was seemed due to the non-adhesive nature of the surfaces. On the other hand, L929 could adhere well on the $2C_{18}$-Gly-OH, $2C_{18}$-D-Glu-C_2N^+, and $2C_{18}$-SUC-SO_3^- Na^+ films. One possible reason of those phenomena was the alternation of the activity of adsorbed proteins. Hamachi et al. presented that myoglobin was activated by adsorbing on the synthetic lipid film.[13] Same as their result, the serum proteins adsorbed on the above three lipid films might have some activation activity for cells or other serum proteins.

It becomes clear that DPPC, DPPS, and DPPE films are good substrates for L929 cells. It seemed that proteins adsorbed on these three lipid films had same activities as those on TCPS. The reason of the difference of the HSP70B mRNA expression in the cells adhered on to lipid films are unclear at present, however, some possible suggestions were reported by other researchers. It was reported that DPPC interacted with some coagulation factors by hydrophobic interaction.[4,5] Additionally, species and amount of adsorbed proteins, [14] and alternation of the activity of adsorbed protein[13] are also key factor for cell-lipid film interaction. We showed that there were some unknown interaction between lipid films and L929 cells. Next goal of our study is to clarify the factors affecting those interactions.

References and Notes

1 K. Kobayashi, K. Takaoka, and S. Ochiai, *Thin Solid Films*, **159**, 267 (1988).
2 A. Fischer and E. Sackmann, *J. Colloid Interface Sci.*, **112**, 1 (1986).
3 N. K. Adam, "The Physics and Chemistry of Surfaces," 3rd ed, Oxford University Press, Oxford (1941).
4 E. H. Ellison and F. J. Castellino, *Biophys. J.*, **72**, 2605 (1997).
5 J. S. Atkins and P. R. Ganz, *Mol. Cell. Biochem.*, **112**, 61 (1992)
6 T. Sato, M. Endo, and Y. Okahata, *J. Biomater. Sci., Polym. Edn.*, **7**, 587 (1995).
7 A. Kishida, S. Kato, K. Ohmura, K. Sugimura, and M. Akashi, *Biomaterials*, **17**, 1301 (1996).
8 S. Kato, T. Akagi, A. Kishida, K. Sugimura, and M. Akashi, *J. Biomater. Sci., Polymer Edn.*, **8**, 809 (1997).
9 S. Kato, T. Akagi, K. Sugimura, A. Kishida, and M. Akashi, *Biomaterials*, **19**, 821 (1998).
10 Y. Tamada and Y. Ikada, "Cell attachment to various polymer surfaces," in: "Polymers in Medicine II," ed by E. Chiellini, P. Giusti, C. Migliaresi, and L. Nicolais, Plenum Press, New York and London (1986).
11 A. Kishida, H. Iwata, Y. Tamada, and Y. Ikada, *Biomaterials*, **12**, 786 (1991).
12 The RNA was isolated from L929 cells by an acid guanidine method and reversibly transcribed to cDNA. The amplification of cDNA by PCR was done following the previously described procedure:[9] The relative expression was evaluated by the detection of 162 bp band for HSP70B visualized by ethidium bromide staining, and were quantified by image-analysis (SigmaGel, Jandel Scientific Software, U.S.A.) with referring 566 bp band (β-actin cDNA) as an internal standard. Normally, the amplified HSP70B cDNA was fractionated by poly(acrylamide) gel electrophoresis (PAGE), however, agarose gel was used in this study to simplify the experimental process. The good separation of HSP70B cDNA in agarose gel electrophoresis was ensured by comparing that of PAGE.
13 I. Hamachi, S. Noda, and T. Kunitake, *J. Am. Chem. Soc.*, **113**, 9625 (1991).
14 "Surface and Interfacial Aspects of Biomedical Polymers, Volume 2. Protein Adsorption," ed by J. D. Andrade, Plenum Press, New York and London (1985).