Metabolic expression of methacrylate-derivatized sialic acids and surface modification on living cells

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Introduction: Membrane substances at the mammlian cell surface are consisted of proteins, lipids, and sugar chains anchored to the phospholipid bilayer. *N*-acethylneuraminic acid, the most common sialic acid, exsists at the termini of sugar chains on the living cell surface is widespread in cell membrane. Sialic acids are biosynthesized from *N*-acethylmannosamine (ManNAc). It has been reported that unnatural mannosamine derivatives, in which the *N*-acethyl group of ManNAc was substituted with *N*-levulinoyl, are converted to the corresponding sialosides, *N*-levulinoyl sialic acids.[1] We hypothesized that the cell surface modification through metabolic pathway might be useful for preparing novel biofunctional polymers. Methacryloylmannosamine (ManMA) as a mannosamine derivative then was newly synthesized to introduce polymerizable groups on a living cell surface. Here, we present metabolic delivery of methacrylates on a living cell surface and polymerization of the functional groups under cell culture condition.

Experiments:

Materials. ManMA and 2-biotinamidoethyl methacrylate (BiMA) were synthesized according to the modified method reported previously. [2][3]

Preparation for cell culture. We used HeLa and HL-60 cells as cultivate cells in this study. Both types of cells were harvested at 37 ℃ in each appropriate medium under a humidified atmosphere of air containing 5% CO_2.

Surface modification of HeLa cells. HeLa cells (1.0×10^5 cells/ml) were seeded on the tissue culture plates and incubated in the 10% FBS/D-MEM containing of ManMA (10-30 mM) for 3 days. Cells were then washed with phosphate buffered saline (PBS). PBS containing 2-methacryloyloxyethyl phosphorylcholine (MPC) was soaked on the culture plate and redox polymerization was performed at room temperature. After polymerization, cells washed with PBS. The expression of methacrylates on the cell surface was confirmed by fluorescence staining with Alexa Fluore 488 conjugated avidin (5.6μg/ml) for 15min. The reduction of the polymerization ability on ManMA-treated cells was also confirmed with sialidase treatment.

Control of HL-60 cells attachment to a substrate.

HL-60 cells as typical floating cells (1.0×10^5 cells/ml) were cultured by the similar way as cultivation of HeLa cells except using RPMI-1640 medium. After redox polymerization, cells were washed with PBS. ManMA-treated or untreated HL-60 cells were resuspended with fresh medium. The cells were again seeded on avidin-immobilized substrate and stained with Calcein-AM.

Results and Discussion: The cytotoxicity of ManMA was not observed when the concentration was below 30 mM. Figure 1 shows fluorescence micrographs of HeLa cells, which are in contact with fluorescence probes. The cells observed in Figure 1(a) were incubated with ManMA and polymerized with MPC and BiMA. Every cell was homogeneously stained with Alexa Fluor 488 conjugated avidin. This result indicates that the polymer containing biotin exists on the every cell surface. On the other hand, any fluorescence image for cultured cells was not observed in Figure 1(b). The cells in Figure 1(b) were treated with sialidase before redox polymerization. The polymerizable groups expressed on cell surface were disappeared by sialidase treatment. According to the cell culture experiment, it was clarified that the methacrylate groups are expressed on a cell membrane by treatment with ManMA.

HL-60 cells were typical floating cells and the expression of methacrylates on the cell surface was determined by contact with an avidin-immobilized substrate. After redox polymerization with MPC and BiMA, ManMA-treated HL-60 cells were attached on surface with maintaining their round shape. On the other hand, the untreated cells were not attached on the avidin-immobilized substrate even after polymerization. Methacrylates can be expressed on various types of cells and polymerized in ambient condition.

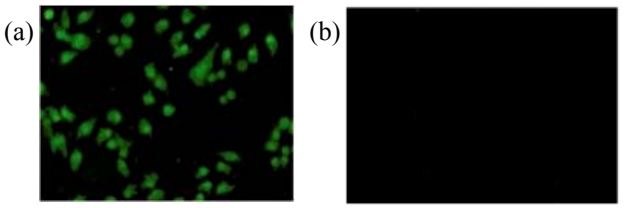

Fig. 1 Fluorescence micrographs of surface modified HeLa cells after contact with fluorescence-labeled avidin. (a) ManMA-treatment and redox polymerization, (b) ManMA-treatment, sialidase-treatment and redox polymerization.

Conclusion: This work describes a new methodology for surface modification of living cells through the polymerization of metabolically expressed methacrylates on living cell membrane surface. The uncanonical functions such as, molecular recognition and cellular attachment can be achieved to the mammalian cells through this metabolic expression. Because this method is tolerant in various types of cells, polymers having different function contributed from the each cell can be also obtained. The biofunctional polymers having cell membrane residues may be applicable for high performance biosensor and target directing drug delivery system.

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