Selectin-mediated cell adhesion on glycoprotein-conjugated hydrogels

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Introduction: Carbohydrates located on the outermost surface of the cell membrane contribute to various forms of communication between living cells and their environment. Recently, the biological and therapeutic functions of carbohydrates have been clarified and many glycopolymer bioconjugates have also been designed. Although these approaches are very successful for use in the regulation of cell-material interaction, almost all synthetic glycopolymers have simple carbohydrate residues because the structure of natural carbohydrates is very complex. In addition, some may not interact uniquely with specific cells and instead show affinity to a broad range of substrates. In the current study, synthetic substrates incorporating metabolic engineered glycoproteins of mammalian cells were prepared for the first time and selectin-mediated cell adhesion on the surface of synthetic hydrogels was demonstrated.

Methods: *N*-Methacryloylmannosamine (ManMA) was synthesized by a previously described method [1]. Other reagents and solvents available in extra-pure grade were obtained commercially and used without further purification.

The concentration of HL-60 was adjusted to 2 x 10^5 cells/mL. The cell suspension (1 mL) was poured into a 24-well tissue culture dish and 110 μL of 50 mM ManMA/PBS was added to the wells. After cultivation for 3 days, the cells were washed three times with fresh medium to remove any free ManMA.

A suspension of ManMA-treated or untreated cells (3.0 x 10^5 cells/mL, 1 mL) was transferred to a 1.5 mL-plastic tube and the cells were disrupted by ultrasonication. The suspension was centrifuged and the precipitate was rinsed three times with PBS. After the supernatant was removed, lysis buffer (100 μL) was added to the tube. The lysate (50 μL) was freeze-dried and mixed with 50 μL of MPC/RIPA lysis buffer. Ammonium peroxodisulfate (APS) in PBS and *N*, *N*, *N*', *N*'-tetramethylethylenediamine was then added to the tube. The molecular weight increment of the glycoprotein via polymerization was determined by Western blot analysis.

Preparation of hydrogels containing glycoproteins: Hydrogels were prepared by a method reported by Kiritoshi et al [2]. MPC/RIPA lysis buffer (2.5 mol/L, 150 μL), MBAA, which is 0.1 mol% of the MPC, APS in PBS (0.22 mol/L, 9 μL) and 3 μL TMEDA were mixed with freeze-dried lysate in a 1.5 mL-plastic tube. The final concentration of proteins in the monomer solution was 1000 μg/mL. Proteins and carbohydrates incorporated in the hydrogels were stained by using Coomassie Brilliant Blue (CBB) staining solution and Periodic acid–Schiff (PAS) stain kit, respectively.

A suspension of HUVECs was introduced into upCell cultureware and cultured until 80% confluent. IL-1α was introduced to the cultureware and stored for 3 h and the dish was then cooled to inertly detach the sheet of adherent cells. The concentration of the recovered cells was adjusted to 1.0 x 10^5 cells/ml and the suspension was in contact with the hydrogels prepared with lysate of HL-60 for 30 min. After contact with the HUVECs, the surface of the hydrogels was observed by phase-contrast microscopy.

Results: The cells were then incubated for 3 days to deliver methacryloyl groups to the glycoproteins of the cells. Redox radical polymerization of methacryloyl functionalized glycoproteins with MPC and a crosslinker resulted in a transparent hydrogel. Fig. 1A and 1B show photographs of MPC hydrogels after staining with CBB and PAS reagents, respectively. The hydrogels prepared with untreated HL-60 cell lysate did not exhibit staining after treatment with CBB and PAS reagents. In contrast, the dyes remained in the hydrogels prepared with ManMA-treated HL-60 cell lysate. The surface density of P-selectin glycoprotein ligand-1 (PSGL-1) on the hydrogels was also detected by gold-colloid-labeled immunoassay. The density of IL-1α stimulated HUVECs adhered on the hydrogels is shown in Fig. 2. On the MPC hydrogels prepared with lysate of untreated HL-60 cells (without ManMA-treatment), very few IL-1α stimulated HUVECs were observed. In contrast, a large number of IL-1α stimulated HUVECs adhered to the hydrogels prepared with the lysate of the ManMA-treated HL-60 cells.

Conclusions: We succeeded in preparing a hydrogel bearing natural glycoproteins of leukemia cells and the selectin-mediated adhesion of cytokine-stimulated endotherial cells on the hydrogel was also observed. In living system, selectin-mediated cell adhesion is considered an essential step in leading to inflammation, reperfusion injury, rheumatoid arthritis, metastasis, infection, etc. Thus, novel synthetic materials, which regulate these bioresponces, could be obtained. The metabolic oligosaccharide engineering with ManMA is robustness for creating biointeractive synthetic materials because this is the first effort to use glycoproteins as monomers for conventional radical polymerization, which is the most practical polymerization method.

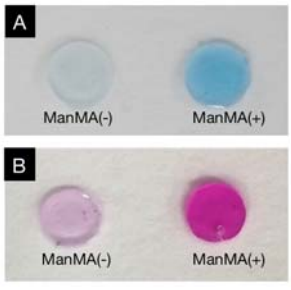

Fig.1 Photographs of hydrogels stained with (A) CBB and (B) PAS.

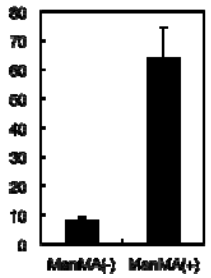

Fig.2 Density of adherent IL-1α treated HUVECs adhered on hydrogels.

References: [1] Iwasaki Y et al., Macromol Biosci 2011;11:1478. [2] Kiritoshi et al., J Biomater Sci Polym Ed 2002;13:213. [3] Iwasaki Y et al., Bioconjugate Chem 2014;25:1626.