

TEMPORAL AND SPATIAL CONTROL OF CELL-GLYCOPOLYMER SURFACE INTERACTION: REGULATION OF HEPATOCYTE CELL SIGNALING BY GLYCOPOLYMER

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INTRODUCTION: Cell adhesion to extracellular matrixes (ECMs) plays pivotal role in a wide of variety of biological process such as proliferation, differentiation and metastasis in animal tissue. Synthetic biomimetic materials have been designed to stimulate cell adhesion and specific cellular functions in tissue engineering [1]. Cell adhesion to materials is initialized by cell surface molecules, while cooperating with material surface molecules through biological recognition or non-specific interaction. Carbohydrate-mediated cell recognition has been applied to enhance selective interactions between the materials and cell surface. We initially developed the carbohydrate-derivatized polystyrenes, poly-(N-p-vinylbenzyl-4-O- β -D-galactopyranosyl)-D-gluconamide (PVLA) and poly-(N-p-vinylbenzyl-6-O- α -D-galactopyranosyl)-D-gluconamide (PVMEA), that have multivalent galactose moieties. The polymers were excellent in mammalian primary hepatocyte recognition mediated by asialoglycoprotein receptor (ASGPR) of the cell surface [2]. Recently, we designed a novel glucose-derivatized polystyrene, poly-(N-p-vinylbenzyl-D-glucuronamide (PV6Gna), which also is recognized by ASGPR [3]. In this study, we used the glycopolymers as a cell adhesion matrix to control integrin-mediated cell signaling, elucidating the events that take place at surface or interface of biological system and synthetic materials.

METHODS: *Preparation of glycopolymers* Amphiphilic sugar-carrying polystyrenes were synthesized by a simple method which couples glycolactone with N-p-vinylbenzyl amine followed by radical polymerization [4, 5]. Molecular structure of hepatocyte-recognizable glycopolymers, PVLA, PVMEA, and PV6Gna, is shown in Fig 1.

Atomic force microscopy (AFM)

AFM image was taken by a scanning probe microscopy (SPM; NanoScope IIIa, Digital Instruments, U. S. A.). SPM was performed in air with an etched 125 μ m silicon cantilever operating in TappingTM mode with a scan size of 200 nm.

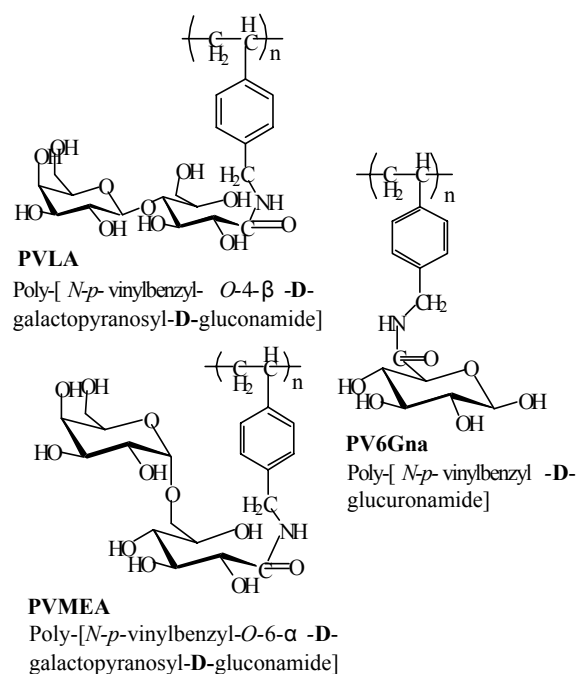


Fig. 1 Molecular structure of glycopolymers

Western blot analysis and immuno-precipitation

Cultured hepatocyte was solubilized in lysis buffer. The lysate was precipitated with primary antibody-conjugated agarose, then the precipitate was separated by SDS-PAGE and analyzed by western blot with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody (Jackson ImmunoResearch Lab.) and the ECL method was used (Amersham Pharmacia Biotech) using standard techniques.

RESULTS: Initial adhesion affinity of hepatocyte for glycopolymer surfaces was increased with an increase of the concentration of coated-PVLA, PVMEA, or PV6Gna. However, the hepatocyte adhesion strength at 30 min after adhesion was not paralleled to initial cell adhesion affinity. Rapid initial adhesion was followed by ASGPR-independent post-adhesion process on glycopolymer surfaces. Atomic force microscopy (AFM) was performed in order to characterize the geometric structure of the coated-PVLA and -PV6Gna. PVLA was coated to PS surface with different geometric structure depending on the coating concentration, as presented Fig. 2. The

post-adhesion process was controlled by microenvironmental distribution of ASGPR-glycopolymer interaction, which had been dependent on geometric structure of coated-glycopolymer surface, as presented in Fig. 3.

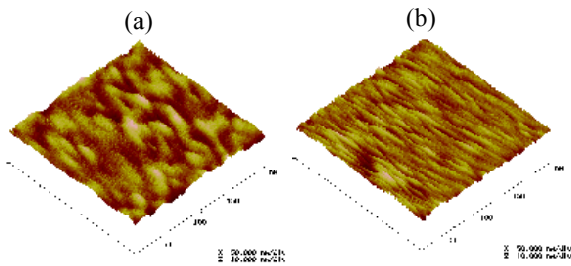


Fig. 2. 3-D AFM image of PVLA-coated PS surface (a) 0.5 μ g/ml, (b) 100 μ g/ml. Yellowish domains point PVLA.

Integrin-mediated signaling, such as cell growth, anti-apoptotic ability, and cell spreading, was restricted in the hepatocyte cultured on the glycopolymer surface, for example on PVLA surface at high coating density, which had induced the clustered ASGPR-glycopolymer interaction at the site of the initial adhesion contact. We suggest that integrin-mediated signaling is a part of post-adhesion processes on the glycopolymer surfaces and can be controlled temporally and spatially by employing glycopolymer surface as an adhesion matrix.

DISCUSSION & CONCLUSIONS: Sugar-carrying polystyrene was a good model for assessment of the role of carbohydrate on hepatocyte cell adhesion to synthetic glycopolymers.

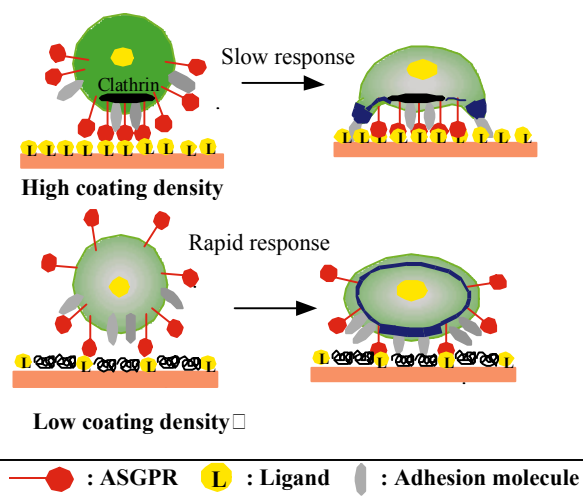


Fig. 3. Illustration of behavior of hepatocyte adhered to glycopolymer surface.

We found a well-defined culture system by controlling hepatocyte adhesion temporally and spatially, while interpreting hepatocyte behavior on the synthetic glycopolymers. These observations could be helpful for basic study of integrin-independent cell signaling for normal hepatocyte and for improved design of cell transplantation devices and cell culture substrate for tissue engineering.

REFERENCES: ¹ J. A. Hubbell (1995) *Bio/Technol.* 13: 565-576. ²K. Kobayashi, et al. (1994) *Carbohydrate-containing polystyrene in Neoglycoconjugates* (eds Y. C. Lee and R. T. Lee) Academic Press 262-282. ³S.-H. Kim, et al. (2001) *J. Biol. Chem.* In press. ⁴K. Kobayashi, et al. (1983) *Polymer J.* 15: 667-671. ⁵S-H. Kim, et al. (2000) *Biotech. Lett.* 22: 1049-1057