Initial Cell Adhesion on RGD-immobilized Phospholipid Polymer Brush Layer with Different Molecular Mobility

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Statement of Purpose: The dynamic properties of materials surfaces, including the water structure, molecular chain mobility, and elasticity as molecular assembly, should be considered to control the biological responses because these responses also time-dependently change on the materials surface. In particular, cell adhesion on the materials surface is important because the cells would be quite sensitive to the dynamic properties of the materials surface. The matrix stiffness for differentiated cells, for example, is known to influence focal adhesion structure and the cytoskeleton [1]. On the other hand, at the initial cell adhesion, cells directly interact with the hydrated protein layer, which has quite low elasticity compared to the materials itself. In this regard, the dynamic properties of the diffusing layer at the surface would affect the initial cell adhesion process. The objective of this study is to analyze the initial cell adhesion from the viewpoint of the dynamic surface properties based on the molecular mobility of dissolving polymer chains. In this study, the elasticity of the diffusing polymer layer at the surface was controlled by three dimensional surface structure of poly(2-methacryloyloxyethyl phosphorylcholine (MPC)) (PMPC) brush layer. To induce the specific interaction with cells, the cell adhesion peptide was immobilized at the outermost surface of PMPC brush layer. The relationship between the initial cell adhesion behavior and mobility of the dissolving polymer chains at the surface was discussed.

Methods: PMPC brush layer with different thickness was prepared at the gold-evaporated glass substrate by using surface-initiated atom transfer radical polymerization method with a free initiator [2]. The terminal bromide group at the grafted PMPC chains was converted into the azide group by using sodium azide. The octapeptide containing the RGD (Arginine-Glycine-Aspartic acid) sequence and alkyne group at both terminal sides was prepared using solid-phase synthesis, and immobilized at the outermost surface of PMPC brush layer by click reaction (Fig. 1). The physicochemical surface structure was confirmed using Fourier transform infrared reflection adsorption spectroscopy and spectroscopic ellipsometer. The adsorbed amount of proteins on the surface from 10% fetal bovine serum (FBS) in phosphate buffered saline (PBS, pH 7.4) solution was quantified with surface plasmon resonance measurement. The elasticity of the surface was quantified from the dissipation energy measured with quartz crystal microbalance with dissipation method [3]. Cell adhesion test was performed using human cervical cancer (HeLa) cells in the culture medium with 10% FBS. After the cell culture for 6 h, the phenotypes of the adherent cells were observed with phase contrast microscopy.

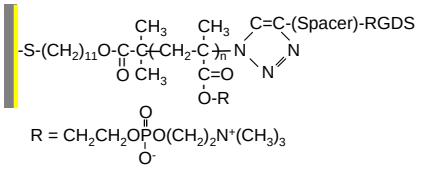

Fig. 1. Chemical structure of RGD sequence-immobilized PMPC brush surface.

Results: From the ellipsometric thickness analysis, the graft density of PMPC brush surface was 0.24 chains/nm^2, indicating the formation of highly dense structure. The dry thickness of PMPC layer could be controlled from 10 nm to 90 nm by the concentration of the free initiator. The dissipation energy from PMPC layer with the thickness of 10 nm and 70 nm was 10.0 x 10^{-6} and 150 x 10^{-6}, respectively, which indicated that the elasticity of the surface in water could be controlled by the length of water-soluble PMPC chains at the surface. The adsorbed amount of proteins from the 10% FBS in PBS solution on the RGD-immobilized PMPC brush surface was less than 10 ng/cm^2, indicating the elimination of any kind of non-specific interaction. Fig. 2 shows the phase contrast microscopic images of the adherent cells on the RGD-immobilized PMPC brush surface with different elasticity. The cells adhered on PMPC brush surfaces with RGD sequence, while cell adhesion was hardly observed on PMPC brush surface without RGD sequence. This result indicated the specific interaction between the integrin and RGD sequence. Almost all adherent cells took a round shape, suggesting that actin fiber would not be firmly formed in a cell. More cells adhered on the RGD-immobilized surface with high elasticity, which would have low mobility of dissolving PMPC chains. This result indicated that the integrin-RGD interaction would be weakened when the mobility of RGD sequence immobilized at the dissolving polymer chain is high.

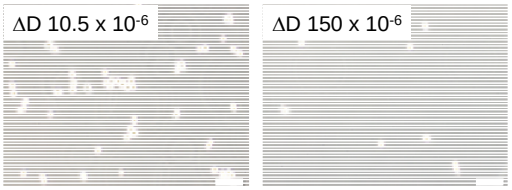

Fig. 2. Phase contrast images of HeLa cells on PMPC brush surface with different elasticity. ΔD means the dissipation energy from the surface. High ΔD value corresponds to low elasticity of the surface. Scale = 100 μm.

Conclusions: The specific cell adhesion on the surface would be affected by the mobility of RGD sequence linked to the dissolving polymer chain at the surface.

References: [1] Engler A. J. Cell 2006;126:677–689. [2] Inoue Y. Colloids Surf. B: Biointerfaces 2010;81:350-357. [3] Inoue Y. Colloids Surf. B: Biointerfaces 2012;89:223-227.