Cytocompatible Polymer Surface with Photocleavable Groups for Regulating the Single Cell Attachment/Detachment

Batzaya BYAMBAA [1], Tomohiro KONNO [1], and Kazuhiko ISHIHARA [1, 2]
[1]Department of Bioengineering, [2]Department of Material Engineering, The University of Tokyo, Tokyo 113-8656, Japan
E-mail: batzaya@mpc.t.u-tokyo.ac.jp

Statement of Purpose: The value of molecular analysis in a single cell needs into existing workflows increases dramatically. A conventional high-throughput (HTP) population based averaging techniques often misleading rare but important events. Study of rare cells (stem cells, progenitor cells) in the field of e.g., Laboratory In a Cell (LIC), single cell gene expression, single cell imaging does not allow HTP population based protocols. Thus attention to the advantages and importance of studying single cells are increasing and a new analysis of individual cell technique is required. The goal of this study is to fabricate a novel substrate, which can regulate cell attachment/detachment selectively by a single cell level for development of bioanalytical devices. For this purpose, we prepared a cytocompatible polymer surface with photocleavable groups to control the cell attachment/detachment using a photochemical reaction. As a polymer, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer bearing photocleavable (PL) monomer unit (PMB-PL) was synthesized. The MPC units are well known as a cytocompatible and nonbiofouling property [1, 2]. The irradiation with UV light removes the cell adhesive molecules on the surface. Thus, cell detachment with UV irradiation may be achieved. We would like to confirm that the photochemical reactions on the polymer surface are powerful for developing the single cell analytical devices.

Methods: The PL unit was methacrylate with 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitro-phenoxy] butyric acid. Characterization of PMB-PL modified glass-based substrate was analyzed by absorption spectroscopy, X-ray photoelectron spectroscopy (XPS) and contact angle measurement by water. A cell suspension (1.0×10^4 cells/mL, 2 mL) was seeded on the surface and incubated under 5% CO_2 at 37ºC. After 2 h of incubation, unattached cells were washed off with medium and UV light (λ=360 nm, 80 mW/cm^2) irradiated to the surface for several periods. Cells detached by photoirradiation were recovered and calculated for cell number.

Results: Photoreaction of PMB-PL was followed by absorption spectra. Before irradiation, polymer solution had absorption peaks at 300 nm and 343 nm. After photoirradiation, it was decreased at 343 nm and increased at 263 nm peaks, which are corresponded to photoinduced cleavage of ester group in the PL unit. The PMB-PL modified glass-based substrate showed similar absorptions as the solution which indicates the polymer kept its photochemical activity on the surface-immobilized state. The PMB-PL coated surface had a phosphorus peak, a nitrogen peak, an oxygen peak, a silicon peak, and strong carbon peak by XPS. After 60 sec photoirradiation of modified surface, the ratio of P/C increased and the ratio of N/C, O/C decreased. These results indicated to the notion of photoinduced cleavage of ester group by a photoirradiaton on the PMB-PL modified surface. After modification of PMB-PL to the silica substrate, the surface contact angle by water changed from 50° to 88° which means coated surface is more hydrophilic than silica board while PMB surface is hydrophobic. After 10 min photoirradiation of PMB-PL surface, the water contact angle increased at 101°. The increasing of contact angle reflects the photorelease of PL units from the surface and remaining of hydrophobic PMB units. However, the contact angle after 10 min irradiation (101°) was still lower than that of PMB surface (112°) which imply that photochemical reaction of ester group was not complete. Thus, it can be control the surface chemical composition by this process.

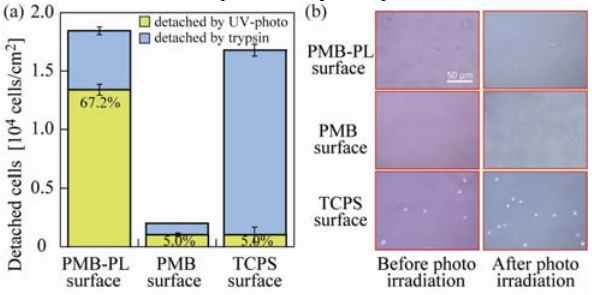

Fig. 1 (a) Detachment of attached cells by photo chemical reaction on the surfaces; (b) Phase contrast micrograph of cells on the surfaces.

The photoinduced detachment of cells on different surfaces was examined (**Fig. 1**). Approximately 95% of seeded cells were attached to the PMB-PL modified surface and almost 75% of attached cells were detached by photoirradiation. UV light removes the cells which are bound to PL unit by photochemical reaction and nonbiofouling surface were remained. These results strongly support the detachment of the cells on the PMB-PL modified surface was selectively related to photochemical cleavage of PL unit by photoirradiation. The detached cells after photoirradiation were kept their physiological phenomena as before cell attachment. The photocleavage efficiency of cells was approximately 75%. This result implies that the photochemical reaction of ester group was not complete, as described above. These results demonstrate the cell detachment on the modified surface related to the photochemical cleavage of PL unit.

Conclusions: In this study, a novel photocleavable and cytocompatible surface prepared and characterized. Attached cells onto this surface detached using photochemical reaction by single cell level. It is expected this technology can be a powerful tool for a single cell analysis system, and the expansion of this tool can be applied for various cell-based fields such as medical, pharmaceutics, and bioengineering.

References: [1] K. Ishihara *et al.*, *Polym. J.*, **22**, 1990, 355. [2] T. Konno *et al.*, *Biomaterials*, **26**, 2005, 1381.