論 文 の 内 容 の 要 旨

論文題目　**Photoresponsive Biointerfaces for Controlling Cell Adhesion Behavior**

(光応答性バイオインターフェイスの構築および細胞接着挙動の制御)

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Abstract: The overall of this study is the development on understand and control phenomena occurring at the interface between artificial materials and biological molecules. In particular, this study was investigated a novel photoresponsive interface bearing a PC group for regulate its cell adhesiveness. For this objective, the model interface which can robustly regulate its surface properties such as a cell adhesiveness using external stimuli as a light is established and surface characteristics were demonstrated. Based on this surface investigated in this study, it is intended to establish a novel biointerfaces to manipulate to collect the targeted living cells with harmless at a time. The biointerfaces achieved in this study is expected to elicit abundant progress in fundamental cellular studies, cell-based analysis, and well-defined cell engineering.

1. Introduction

The development of dynamic surfaces that can control cell adhesiveness by external stimulus is an extremely essential in biomedical applications, ranging from tissue engineering, cell-based drug screening, to the fundamental biological studies [1, 2]. The purpose of this study is the investigation of a novel biointerfaces those are capable for dynamic controlling of surface-properties including cell adhesiveness using external photo-stimulus. Among the dynamic substrates, the photoresponsive interfaces are specialized because they allow the spatial and temporal resolution control for cell adhesion without addition of any chemical stimulants. Photoresponsive surfaces modulate to alter localized *in situ* pattern of cell adhesion and provide unprecedented opportunities for biological insight to cell-surface interactions and analyzing dynamic cellular activities [3-6]. Although these photoresponsive surfaces present excellent performance on cell adhesiveness on surface, the interest is keen for a higher level of performance for most cell biological studies. One of the most important and considerable performance is the increasing of cytocompatibility of surface. In order to improve abundant cytofunctionality and cytoselectivity, it is important to passivate the material surface from undesired biomolecule interactions. The 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers which have a bioinert phospholipid group are well known to suppress the non-specific biomolecule interactions because of its properties mimicking the outer surface structure of natural living cell membrane [7-9]. With incorporation of biofunctional units, the MPC polymers could form phosphorylcholine (PC) covered surfaces capable of selectively interacting with specific biomolecules.

The objective of this study is the designing and engineering of a novel biointerface which is able to regulate its cell adhesiveness dynamically via the photochemical reaction. For this objective, the material surface was designed with three essential strategies: (i) precise and robust control of cell adhesiveness with a photoprotecting 2-nitrobenzyl group; (ii) passivation of undesirable biomolecules adhesion onto surface with PC unit; (iii) conjugation of desired biomolecules using *N*-hydroxysuccinimide (NHS) group.

In section 2, the preparation and characterization of biointerface were explained. The key factor for designing biointerface is related to effectiveness of photocleavage of photosensitive group while showing high bioinert property. Another important factor on engineering of biointerface is the simplicity of process for effective modification. Additionally, the varieties of surface modification for organizing improved performance are important. From these viewpoints, the photoresponsive phospholipid surfaces were prepared.

In section 3, the control of cell adhesion on engineered surfaces using photochemical reaction was explained. The cell adhesion onto surface is a very complicated process and depends on a range of physical and biological factors. Among them, this study focused on three main factors, those are effect of the surface properties, effect of adsorbed proteins, and effect of adhered cells. For the effect of surface properties, the surface electric property, surface morphology, surface wettability, and surface functional groups were discussed. For the effect of adsorbed protein, the effect of serum in culture medium, and the effect of cell adhesive ligands for targeted cells were discussed. For the effect of adhered cells, the cellular type and initial seeding density were discussed. For the designing of targeted cell adhesion, the epidermal growth factor (EGF)-EGF receptor (EGFR) interaction were used. As a result, the control of cell adhesiveness on engineered surfaces was successfully achieved by photoirradiation. The photoinduced collection rate of adhered living cells was changed by irradiating with a light at certain doses (mJ/cm^2), initial seeding cell density, and density of conjugated ligands. Furthermore, the cellular functions of collected cells were evaluated.

In section 4, the summary of this study was explained.

I believe that the achieved biointerface in this study will provide a promising and useful tool for biomedical applications, such as cellular analysis and fundamental cellular studies.

2. Preparation and characterization of photoresponsive biointerfaces

The polymeric surfaces are most commonly used in biomaterials because they can be easily synthesized to give versatile variation of properties. These surfaces can be prepared using several techniques with different complexity and applicability. One of the simplest techniques of preparing polymer film on any optional substrates is physisorption, e.g., spin coating method. In this study, the polymer surfaces were achieved with the photocleavable phospholipid polymer coating to the glass substrate. The photocleavable phospholipid polymers, the PMB-PLs, which are incorporating cytocompatible PMB [10-12] bearing photocleavable (PL:4-[4-(1-methacryloyloxyethyl)-2-methoxy-5-nitrophenoxy]butyric methacrylate) group were synthesized. The monomer unit composition was quantitatively related to the feeding ratio. From the UV/VIS adsorption spectra, the intensities of adsorption peak at 348 nm were quantitatively related to the composition of PL unit in each polymer. Additionally, the cell adhesion was suppressed on PMB-PL352 modified surface which depends on the MPC unit composition. Therefore, the PMB-PL253 was used in this study to obtain high efficiency of photocleavage of PL unit and sufficient adhered cell numbers on surface (abbreviated as PMB-PL). The coverslip glasses were coated thrice with PMB-PL (3000 rpm, 0.5wt% EtOH.).

In order to determine performance of modified surface, the changes of surface properties during photoirradiation were characterized. Following photoirradiation, the peak intensity at 300 nm and 348 nm was decreased those are related to photocleavage and release of 2-nitrobenzyl ester group from the surface [13, 14]. Thus, it can be concluded that the polymer kept its photochemical activity on the surface modified state. The changes of surface characteristics of PMB-PL surface before and after photoirradiation is examined and discussed. From XPS measurement, the ratio of peak intensity attributed to P_{2p} to C_{1s} was increased during photoirradiation from 0.088 to 0.15, which indicates the photorelease of PL unit from the surface. Following

photoirradiation, the PMB-PL surface was converted to neutral charged and more hydrophilic surface. The ζ-potential of the PMB surface was reported as almost zero [15-17]. From XPS and ζ-potential results, the surface obtained after photoirradiation presents similar surface properties as PMB surface, a consequence of photocleavage of PL unit.

The photocleavable PL monomer unit has a -COOH group in its side chain which supplies variety of surface modifications and provides bioconjugation of desired molecules on the surface. The carboxyl group was converted to the -*NHS* group, which the cell adhesive EGF ligands were subsequently conjugated. To demonstrate the photorelease of ligands on PMB-PL surface, I conjugated FITC (Fluorescein isothiocyanate) labeled EGF on the PMB-PL modified surface. A sharp peak was observed at 516 nm which is derived from existence of FITC labeled EGF on the surface. It was also observed the decrease of fluorescent intensity against different irradiation time which indicates decrease of surface protein density via the photocleavage of PL unit. On the any substrate of choice, *e.g.*, glass substrate, it was able to prepare photoresponsive cytocompatible surface with PMB-PL polymer coating.

3. Control of cell adhesion on photoresponsive biointerfaces

The control of surface cell adhesiveness of prepared surface was evaluated with three measurements, those are number of adhered cells, morphological changes of adhered cells, and number of detached cells. The A431 cells (human epithelial carcinoma cell) were used as a model cell line which overexpresses the EGFR.

To evaluate cell adhesion ability on PMB-PL surface, the number of adhered cells was observed in two different cell culture media, 10% serum-supplied and serum-starved medium. The adhesion rate of A431 cells was distinctly higher in serum-supplied culture. Additionally, it was observed cell detachment rate of adhered cells from PMB-PL surface in serum-supplied and serum-starved culture. As a result, no significant difference was observed between cell detachment rates. To eliminate the complicated side effect of serum proteins on cell adhesion process, the serum-starved condition with 4 h culture time was used in all subsequent experiments.

To develop substrate for targeted cell captures, the EGF conjugated cell culture surfaces were prepared. The cell adhesion rate to the different density of EGF conjugated surface is examined. On the EGF conjugated surfaces, more than 90% of seeded cells were adhered to the surface without varying the local density of EGF ligands. On the other hand, even there was no EGF ligands conjugated on the surface, around 60% of seeded cells were adhered, that the cells were probably bounded to the photocleavable PL unit on the surface. For the control experiment, the albumin conjugated surface was showed almost negligible amount of cell adhesion compared to EGF conjugated surface, which indicates the cell adhesive molecules were blocked with albumin. Additionally, some morphological changes were observed on adhered cells onto surfaces. On EGF conjugated surface, cells were attached with irregular shape to spherical, and flattened and spread to cover as large area as possible. On non-conjugated surface, cells were attached with spherical shapes and showed low ability to flattening and spreading. The mean cell sizes in diameter of adhered cells on EGF conjugated surface were 30% greater than that of non-conjugated surface.

The photoinduced detachment of adhered cells on each surface was successfully achieved. On the cell adhesive EGF conjugated surface, more than 60% of adhered living cells were detached during photoirradiation (365 nm, 80 mW/cm^2, 60 sec). On the EGF non-conjugated surface, more than 50% of adhered cells were detached. Meanwhile, a very few amount of adhered living cells were detached by photo irradiation on the PMB surface which do not contain photoreactive PL unit. When the polymer surface were re-used after the first irradiation, a very few amount of cells were adhered onto it and a negligible amount of cell detachment were

observed. Those results strongly support that the photocleavable units on the surface control cell adhesion site, as evidenced the photoreaction of these units were induced the decrease of cell adhesion rate on the surface. Moreover, it was confirmed that the cellular activities of collected cells from PMB-PL surface were maintained higher cellular functions as same as those seen on unexposed control cells, which indicates PMB-PL surface provides harmless detachment of adhered cells.

4. Conclusions

The photoresponsive surfaces bearing PC groups investigated in this study is a valuable tool to investigate the bioactivity of conjugated biomolecules, and affords a selective mechanism by which specific cells can be recovered from the surface using UV-light. This interface is an excellent strategy for preparing a highly purified cell suspension. The development of the photoresponsive surfaces is important because these platforms provide more findings on biological molecules on the surface and to identify adsorption behavior to the surfaces. It can be expected that the achieved biointerfaces in this study can contribute towards to the cell-based biomedical fields, such as development of cell based analysis technology, cell separation/isolation technology, and as fundamental cellular studies.

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