論文の内容の要旨

論文題目　Cell patterning in microchannel using photochemical reaction
(光化学反応を利用した微小空間における細胞パターニングの研究)

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In this thesis, cell micropatterning method in microchannel was created. Firstly, the strategy of surface modification method combining MPC polymer and PL was demonstrated. Secondly, cell micropatterning using photochemical reaction was developed. Thirdly, cell micropatterning was realized in microchannel.

In chapter 2, glass surface was synthesized by PL and MPC polymer. MPC polymer is known as resistant barrier for biological samples and PL is utilized in selective patterning of biological samples. By combining two compounds, chemicals with different cell adhesiveness surface was realized and estimated. Two different kinds of surface modification methods were performed and the property of MPC polymer-modified surface was confirmed. In 2.2, firstly, MPC polymer was synthesized with PL1 (5-Amino-4-Methyl-2-nitroactophenone) and grafted on activated APTS glass surface (APTS-DSC). The contact angles showed that the surface remained in hydrophobic (45°) even though hydrophilic photolabille MPC polymer (PL1-MPC) was introduced. This result showed that the photolabile MPC polymer was not introduced on the surface, since activity of the hydroxyl groups in photolabile MPC polymer was too low to react with APTS-DSC surface. In 2.3, I altered the surface modification to step-by-step synthesis method. After silanization the glass surface using APTS, photolabile linker and MPC polymer were modified in order. I chose the commercialized Fmoc protected PL (PL2) and coupled to APTS surface and then Fmoc was deprotected. To this surface (APTS-PL), MPC polymer was grafted and the result of contact angle indicated that hydrophilic surface was formed (12°). Furthermore, photocleavage of the MPC polymer was evaluated by UV (365 nm, 150 mW/cm^2) illumination in order to make cell adhesive surface. The contact angle increased up to 3 min of UV exposure and no significance change was confirmed after 3 min. The optimum UV irradiation time was determined to 3 min and adapted to next experiments. Additional surface characterization tools such as XPS and AFM was also used to analyze the modification and elimination of the MPC polymer on the glass surface. The results were in agreement with my expectation. The developed surface modification methods and photocleavage conditions of MPC polymer were applied in micropatterning of biological molecules in next chapter.

In chapter 3, micropatterning of the biological samples using photochemical reaction was verified on glass surface. In 3.2, reduction of the non-specific adsorption on MPC polymer modified surface was demonstrated. The amount of adsorbed proteins (BSA and fibronectin) and cells (MC-3T3 E1) were evaluated and compared to other modified surface. To the MPC polymer grafted surface, 0.22 μg/cm^2 of BSA, 0.05 μg/cm^2 of fibronectin was adhered. Compared to APTS and

APTS-PL surface, the amounts of adsorbed proteins were reduced to 10-14 %. However, after UV illumination, adsorbed proteins (2.12 μg/cm^2 of BSA, 0.43 μg/cm^2 of fibronectin) were recovered to almost same amounts as those of APTS (2.28 μg/cm^2 of BSA, 0.52 μg/cm^2 of fibronectin), APTS-PL surface (2.28 μg/cm^2 of BSA, 0.36 μg/cm^2 of fibronectin). In the case of attachment rate of the cells, there was no cell had observed on MPC polymer modified surface. In contrast, on UV irradiated surface, 212 cells were attached in square millimeter, which showed that cell adhesiveness was recoved by photochemical reaction. In relation to UV exposed surface, cell attachment rate and cell proliferation rate were compared with ECM proteins such as fibronectin, poly (lysine)-matrigel coated surface. The results indicated that no significant changes were observed in 3 different surfaces.

In 3.3, firstly, micropatterning of proteins and cells were demonstrated by UV exposure through the photomask. After selective removal of MPC polymer, FITC-BSA was attached on UV irradiated area and fluorescence was observed (200 μm wide stripes). For MC-3T3 E1 cell patterning, two different sizes of photomasks (200 μm, 100 μm wide stripes) were used and localized on UV irradiated region. In addition, photomask having 40 μm wide stripes was used and ECs were patterned on UV illuminated region as same size as photomask. To verity single cell level patterning, round shaped photomask with 30 mm spots were used, which resulted in single cell was attached on one spot of pattern. Secondly, isotropic tendency of the localized MC-3T3 E1 cells on pattern size was investigated. Three different conditions were used, which were without a photomask, 200 μm and 70 μm wide stripes of photomask. The cells were placed by each different conditions and a part of adhered cells (140 μm × 100 μm) were observed. No isotropic direction was seen in the case of without a photomask and 200 μm wide stripes, except in 70 μm wide stripes.

Finally, two different types of cells were patterned on the same glass surface. After placing 1st cell type (MC-3T3 E1) cells, UV was illuminated to 900 μm distance from 1st patterned cells, then 2nd type cells (ECs) were seeded and ECs were introduced on 2nd UV exposed area. I succeeded in positioning two different types of cells on the same glass surface by photochemical reaction.

In 3.4, the stability of micro-patterned cells was demonstrated by culturing of MC-3T3 E1 cell patterns (width 100 μm stripe) for 5 weeks and ECs cell patterns (width 40 μm stripe) for 3 weeks on each glass surface. They maintained the micropatterns in safe for long-term culture. These results indicated that MPC was chemically bonded in safe to glass surface and not affected by synthesized and secreted proteins from the patterned cells during the cultures.

In chapter 4, cell micropatterning using photochemical reaction was adopted in microchannel. In 4.2, cell micropatterning conditions were set up and realized on the glass surface in chapter 2, 3. These conditions should be re-tested whether it can be applied in microchannel directly or not. Firstly, I evaluated the UV transparent on microchip which was made by quartz plate. The model system was demonstrated by inserting quartz plate with the same thickness as microchip between MPC polymer modified glass surface and photomask. In results, cell micropatterning was successful and no significance difference was found on micro-patterned cells. This result was in agreement with former results in chapter 3.3. Secondly, the rate of cell adherence

on UV illumination time was confirmed. UV irradiation time was varied to 1, 3, and 5 min, and the cell suspension was introduced into microchannel. After 2 h of incubation, 35 cells, 266 cells, and 246 cells were immobilized on each UV exposed surface. After 3 min, the cell attachment rate reached a plateau; no significant difference was occurred. These are in good accord with the results in chapter 2.3.2. Thirdly, the concentration of MC-3T3 E1 cell suspension to introduce in microchannel was optimized. The concenetration of the suspension was varied to and cell culturing in microchannel was observed. When the concentration of cell suspension was higher than 1×10^7 cells/mL, cells could not spread out onto the micochannel surface, because of the aggregation of cells. In the flow condition of medium, most of the cells were flusheded out. It was clearly proved that optimum concentration of cell suspension was $5\sim8 \times 10^6$ cells/mL.

In 4.3, based on re-estimated experimental conditions in chapter 4.2, cell micropatterning was performed in microchannel. The MC-3T3 E1 cells were adhered to photochemically micro-patterned region, after 2 hr of incubation, the micro-patterned cells were found to be flowed out under flow condition. The cell aggregation is supposed to make cells out of the microchnnel. To reduce the risk, cell attachment rate should be also confirmed before introducing the cell suspension. Cells are likely to make aggregation when they are seeded on non-biofouling surface. When cell densitiy was reduced to 4×10^6 cells/mL according to cell adhesive area, cells were patterned in safe on UV exposed area after 24 h of incubation. The cell adhesive area is also important point to be considered for cell micropatterning in microchannel. Finally, ECs and MC-3T3 E1 cells were placed in same microchannel using photochemical reaction. The Distance of cells was regulated by 200 μm.

In 4.4, the stability of micro-patterned ECs (width 200 μm stripes) was observed in microchannel. In the flow system, patterned cells were maintained the patterns in safe for 14 days of culture. MPC polymer was stable enought to inhibit the cell migration out of the patterns.

Finally, in chapter 5, conclusions and future perspectives are described.