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**[P005] Poster**

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# [P03-427] Preparation of photodegradable hydrogel surfaces with a cell-trapping moiety for single-cell analysis

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**[Purpose]**
Cell analysis techniques to investigate cellular phenotypes, such as morphology, motility, gene expression, and cell-cell interactions, have been essential for elucidating biological phenomena and for diagnosing and treating diseases. However, important minor cells involved in biological phenomena may often be overlooked when they are homogenized by other major cells in conventional cell analysis methods. Therefore, in recent years, technologies for single-cell analysis have become increasingly important. Hitherto, single-cell separation techniques using microwells, microstructures, droplets, and other methods have been widely used. However, in the case of adherent cells, conventional methods are difficult to use to retrieve specific cells and investigate their phenotypes because the restricted microenvironments prevent them from adopting their native, spread morphology. In addition, once these cells are attached to the substrate, such as a glass-bottom dish, they are difficult to collect without cell damage. Namely, mechanical or enzymatic detachment often causes cell destruction or loss of genetic material. Consequently, the accuracy of subsequent single-cell analysis decreases. Therefore, in this study, we designed a two-layer photoresponsive hydrogel surface consisting of a photoactivatable cell-trapping moiety and a photodegradable hydrogel for efficient cell retrieval.
**[Method]**
We formed hydrogel surfaces on a glass substrate by reacting the succinimide group of photodegradable 4-arm PEG and the amino group of gelatin. Subsequently, we modified the photoactivatable cell-trapping moiety on the hydrogel surfaces. In this experiment, the trapped adherent cells could be observed over time by microscopy as they adhered to the hydrogel layer surface, changed their morphology, and moved freely from the trapping spots. Subsequently, a specific single cell could be precisely collected using a microcapillary through localized hydrogel degradation by second light irradiation. These single cells were subjected to gene analysis, such as single-cell real-time quantitative PCR and single-cell RNA sequencing.
**[Results]**
Using such a molecular design, it is possible to arrange single cells at high density with uniform intervals by light irradiation. Therefore, the individual cells did not become excessively close to one another on the hydrogel surface, even at high density, unlike in random cell seeding. Namely, intercellular contact between adjacent cells was prevented during monitoring, allowing for individual cell collection. As a result, this method enabled high-throughput monitoring of morphological changes and gene expression levels in the individual cell.
**[Conclusion]**
We demonstrated that it is possible to identify heterogeneity among invasive model cancer cells by linking dynamic morphological features and gene expression levels on every single cell.