細胞膜裏側の定量的 1 細胞解析のための細胞膜シートアレイの開発

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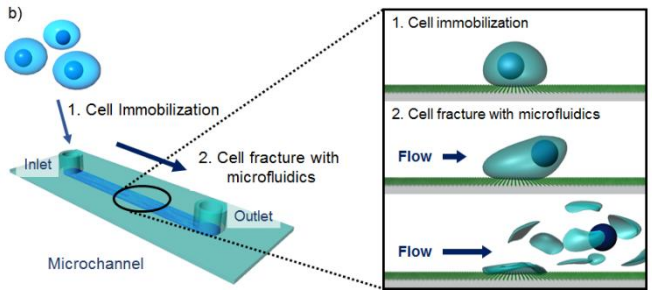
Cell Membrane Sheet Array for Quantitative Single-cell Analysis of the Cytoplasmic Face of Plasma Membranes ([1]*Graduate School of Engineering, The University of Tokyo,* [2]*Research Center for Advanced Science and Technology (RCAST), The University of Tokyo,* [3]*St. Luke's International University,* [4] *Okayama University of Science*) ○ Yuki Umeda,[1] Satoshi Yamaguchi,[2] Shinya Yamahira,[3] Motonao Nakamura,[4] Akimitsu Okamoto[1,2]

The molecular network on the cytoplasmic face of the plasma membrane is an important research target involved in a wide range of biological phenomena and diseases. However, different from molecules on the cell surface, it is difficult to visualize and control their functions by treating with antibodies or inhibitors. Therefore, to expose the cytoplasmic face of the cell basal membrane, methods that disrupt the roof membrane of the cells has been developed by using sonication or other means[1]. We also developed a method that uses microfluidic shear stress to instantly break cells into a sheet-like cell basal membrane, followed with analysis of the exposed cytoplasmic surface (Figure)[2]. In this study, we developed a cell membrane sheet array by applying microfluidic flow to a single-cell array on the photoactivated PEG-lipid surface. Mouse leukocyte BaF3 cells were arrayed by irradiating an array pattern of circular light (14 μm in diameter), followed with flashing a physiological buffer. As a result, an array of uniform circular cell membrane sheets was prepared with high conversion efficiency (74%). It is expected that the post-translational modifications on the intracellular domain of membrane proteins can be quantitatively analyzed at a single-cell level on this array.
Keywords : Single-cell analysis; Cytoplasmic face of plasma membranes; Microfluidics

細胞膜の細胞質側表面の分子ネットワークは幅広い生命現象や疾患に関わるが、細胞表層の分子と同じように、抗体や阻害剤を作用させて可視化したり機能制御したりするのは困難である。そこで、超音波処理などによって細胞上面の細胞膜を破壊し、底面膜の細胞質側表面を露出させる方法が開発されてきた [1]。我々もマイクロ流体のせん断応力を用いて瞬時に細胞を切断してシート状の細胞底面膜に変換し、露出した細胞質側表面を解析する技術を開発した（下図）[2]。本研究では、光活性化 PEG 脂質表面を用いて並べた 1 細胞アレイにマイクロ流体を作用させ、細胞膜シートアレイを開発した。円形光（直径 14 μm）のアレイパターンを照射してマウス白血球 BaF3 細胞を並べ、高速で生理緩衝液を流すと、高い変換効率（74%）で均一な円形状の細胞膜シートのアレイが調製できた。本アレイ上で膜タンパク質の細胞内部での翻訳後修飾を網羅的に 1 細胞定量解析できると期待される。



1) Saka, *et al.*, *Nat. Commun.* **2014**, *5*, 4509; 2) Izuta, *et al.*, *Sci. Rep*. **2017**, *7*, 14962.