Microfluidic cell unroofing for *in situ* molecular analysis of intracellular biomembranes without permeabilization

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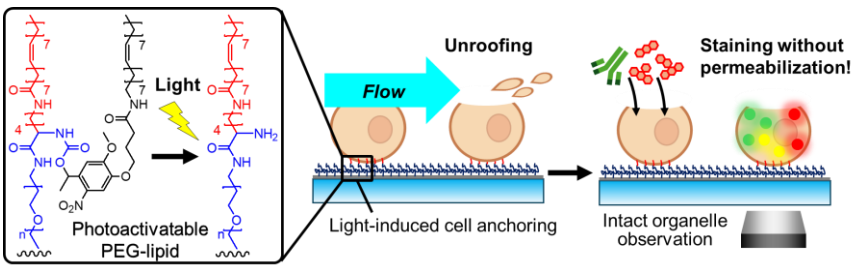
Elucidating the molecular networks on organelle membranes is essential for molecular biology and drug discovery. However, the nature of plasma membrane as a barrier to various analytical tools, including antibodies, makes it challenging to examine intact organelle membranes without affecting their structure and functions via membrane permeabilization. In this study, we aimed to develop a method for unroofing cells and observing the intrinsic membrane molecules on organelles. We previously developed a microfluidics-based cell fracture method to expose the cytoplasmic surface of the plasma membrane.[1] In this method, cells are randomly anchored to the bottom of a microchannel, and laminar flow exerts horizontal stress on the cells, leaving only the bottom plasma membrane with its cytoplasmic surface exposed. This method facilitates the direct examination of biomolecules on plasma membranes but not those on organelle membranes, as organelles are removed by laminar flow.

Recently, we developed a method for precisely arraying single cells on the surface in a light-guided manner using a photoactivatable cell-anchoring material.[2] Using this method, pro-B cell line, BaF3 cells were arrayed on the bottom surface of microchannel with precisely controlled cell intervals. As a result, at sufficiently short intervals, horizontal stress generated by laminar flow instantly fractured the upper cell membranes, without affecting the organelles inside the fractured cells. Subsequently, the nucleus and other organelles in unroofed cells were observed by confocal microscopy and scanning electron microscopy. Notably, mitochondria retained their membrane potential after unroofing, which was completely abolished via conventional permeabilization. Furthermore, the distribution of the mitochondrial membrane protein was successfully observed via immunostaining without permeabilization. Overall, the established cell unroofing method shows great potential for examining the localization, functions, and affinities of proteins on intact organelle membranes.[3]



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