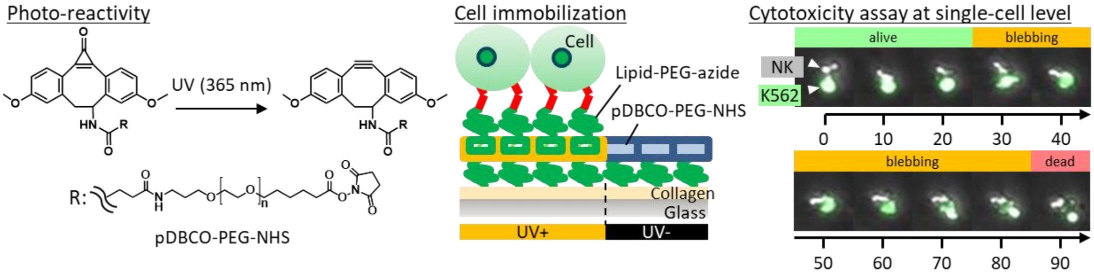
Single-cell analysis of immune cell cytotoxicity using a photo-reactive surface

([1]*School of Engineering, The University of Tokyo*, [2] *Research Center for Advanced Science and Technology, The University of Tokyo*, [3]*St. Luke's International University*) ○ Takahiro Kosaka,[1] Satoshi Yamaguchi,[2] Shinya Yamahira,[3] Akimitsu Okamoto,[1, 2]
Keywords: Photo-reactive; Click reaction; Single-cell analysis; Cell immobilization; Cancer immunology

Cancer immunotherapy is one of the promising medical treatments, which harnesses the cytotoxic interaction of immune cells to cancer cells. It shows high therapeutic effects on some patients, but there are some remaining problems such as non-responders and severe side effects. Considering the heterogeneity of immunocytes and cancer cells, it is critical to analyze their interactions at the single-cell level for the prediction of therapeutic response and the screening of therapeutic cells. However, there are only a few reports for analysis of intercellular communication at the single-cell level. In this research, we aimed to develop the method to create pairs of cells on a substrate, and observe the interaction between immune cells and cancer cells at the single-cell level.

To create the area for cell immobilization by light irradiation, a precursor of dibenzocyclooctyne (DBCO) was employed for light-guided modification of cell-attaching molecules. This moiety is converted to DBCO by UV light irradiation at 365 nm.[1] A compound that has a DBCO precursor at the end of polyethylene glycol (PEG) was designed and modified on a collagen-coated glass substrate. As a cell-attaching molecule, PEG-Lipid was employed and azidated to react with DBCO. It was confirmed that heterogenous cells were patterned at the single-cell level in a light-guided manner by using the photo-reactive substrate and azidated PEG-Lipid (Lipid-PEG-azide).

We fabricated single-cell pairs of NK cells and K562 cells with this system and observed the cytotoxicity of NK cells at the single-cell level. The NK cells caused the different types of target cell death; apoptotic and necrotic cell death, and it was confirmed that this system enabled to observe the cytotoxicity at the single-cell level.



1) Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769–15776.

