TECHNICAL NOTE

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Immune cell-mediated cytotoxicity: single-cell insights with image cytometry

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Summary

- Xdrop® double-emulsion droplets enable cell-cell interaction assays with single-cell resolution.
- The Xcyto®5 image cytometer offers a high-resolution analysis of immune cell functionality in combination with double-emulsion droplets, unveiling functional cell heterogeneity with precision.

Introduction

Most cell-cell interaction assays, including most immune cell-mediated cytotoxicity assays, are performed as bulk assays that mask the true heterogeneity of a cell population. Xdrop facilitates assessment of multiple functionalities with single-cell resolution, thereby enabling the evaluation of heterogeneity within cell populations. An example of this is the combined cell killing and granzyme B (GzmB) secretion time-course assay, which provides mechanistic insight into the process of cell killing.[1]

The Xdrop single-cell functional analysis technology is based on encapsulation of single cells or pairs of cells into 100 pL double-emulsion droplets (DE50 droplets). The droplets serve as tiny cell incubators during the assay, allowing passage of CO_2 and O_2 while retaining larger molecules secreted by the cells. Secreted molecules rapidly build up to detectable concentrations in the small volume of the droplet, dramatically increasing the sensitivity of the assays.

Here, we demonstrate the analysis of cells encapsulated in DE50 droplets by flow cytometry and image cytometry. The reproducibility and versatility of the double-emulsion droplet technology are evident as both technologies yield highly similar results in simultaneous analysis of cytotoxicity and GzmB secretion.

The combined GzmB and cell killing assay

In this experiment, we used human NK cells and K562 cells as effector and target cells, respectively. Before encapsulation, the NK cells were divided into two cultures. One was incubated with stimulation by 50 ng/ml interleukin 15 (IL15) for 24 h. The other (the control) was incubated for 24 h without stimulation. To facilitate tracking of the cells within the DE50 droplets, the NK cells were stained with CellTrace™ Violet (Conc. 1:5,000), and the K562 cells with CellTrace™ Yellow (Conc. 1:5,000).

The NK and K562 cells were separately resuspended in complete MEM α medium supplemented with 10% OptiPrep™, Incucyte® Caspase 3/7 Red Dye (Sartorius, Conc. 1:500) and 2 µM GzmB FAM-labelled peptide substrate. The peptide substrate fluoresces when it is cleaved by secreted GzmB, resulting in the accumulation of an FAM signal inside the droplets. Caspase 3/7 Red Dye is used to monitor apoptosis. The medium for the stimulated NK cells was supplemented with IL15 to continue NK stimulation.

A standard Xdrop protocol for DE50 droplet generation was used to co-encapsulate the cells in DE50 droplets using an Xdrop Well Insert to prevent pre-encapsulation cell-cell interaction.

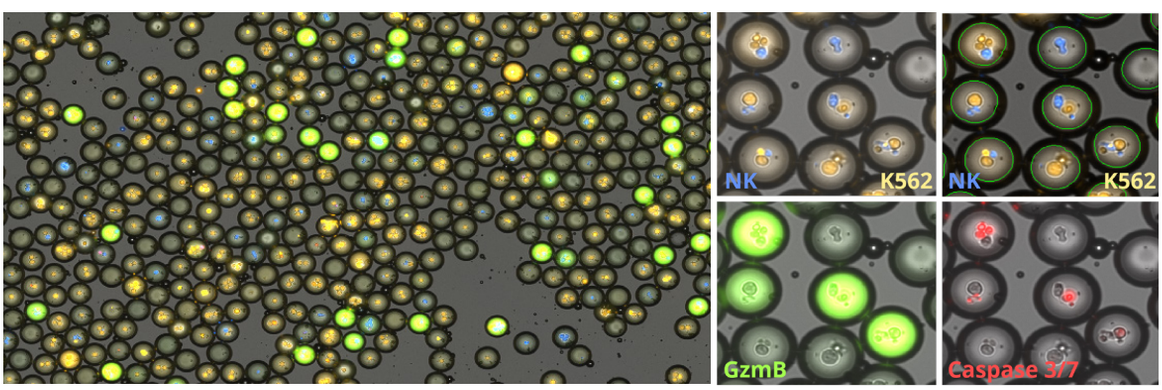

Figure 1. Images captured by Xcyto®5 showcase Xdrop double-emulsion droplets, featuring encapsulated NK cells (blue) and K562 cells (yellow). Assessment of NK cell cytotoxicity includes GzmB secretion (green) and mediation of apoptosis (red). Remarkably, high heterogeneity in GzmB secretion by NK cells is observed, here notable 4 hours post-co-encapsulation with target cells.

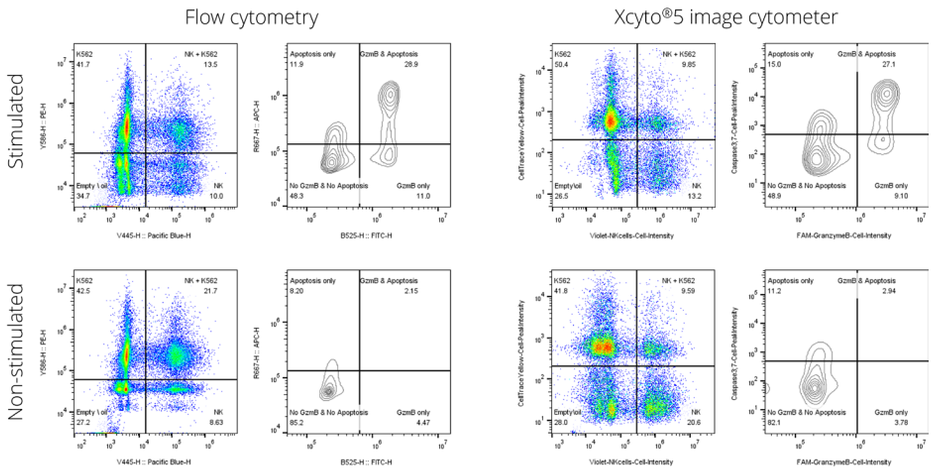

Figure 2. Comparison of data from the Novocyte® Quanteon flow cytometer (Agilent® Technologies) and Xcyto®5 image cytometer. The identified double emulsion droplet populations are shown for each analysis technology: NK+K562 (upper right quadrant), NK only (lower right quadrant), K562 only (upper left quadrant), and empty (lower left quadrant). The contour plots show the GzmB and apoptosis signals detected in the NK+K562 droplet populations.

The cells were incubated within the droplets for 4 h at 37°C in 5% CO_2, and then analyzed using flow cytometry and image cytometry.

Figure 1 shows droplet images acquired on the Xcyto®5 image cytometer 4 hours after co-encapsulation. The stimulated NK cells exhibit significant variability in GzmB secretion, a phenomenon overlooked in standard bulk analyses. Moreover, there's notable diversity in the NK cells' ability to induce apoptosis; some target cells show clear signs of apoptosis, while others seem unaffected by the presence of activated NK cells. These images underscore the critical role of single-cell analysis in comprehending the heterogeneity of immune cell-mediated cytotoxicity.

To validate image cytometry and flow cytometry analyses, immune cell mediated cytotoxicity was assessed side-by-side, as depicted in Figure 2. Both analysis methods identified four distinct droplet populations: NK+K562, NK only, K562 only, and empty (left columns). Analysis of the NK+K562 droplets for apoptosis and GzmB signal (cell killing activity) (right column) shows that GzmB secretion and apoptosis are present only when the NK cells are stimulated with IL15. The consistency between the data obtained from both image cytometry and flow cytometry underscores the robustness of single-cell functional analysis in double-emulsion droplets. This is illustrated in the contour diagrams depicted in Figure 2 and the graphical presentation shown in Figure 3.

Conclusion

Today, the ability to analyze individual cells within a population is paramount in drug discovery, offering more comprehensive and precise insights into cellular diversity.

In this study, we document a detailed single-cell functional analysis of immune cells using double-emulsion droplets as miniature test reactors, evaluated through image cytometry. Xdrop enables the examination of heterogeneity in cell function within cell populations by facilitating single-cell or cell-cell interaction assays. High-resolution analysis is easily conducted using the Xcyto®5 image cytometer.

The versatility of double-emulsion droplet technology is demonstrated by the consistent data produced from both image cytometry and flow cytometry.

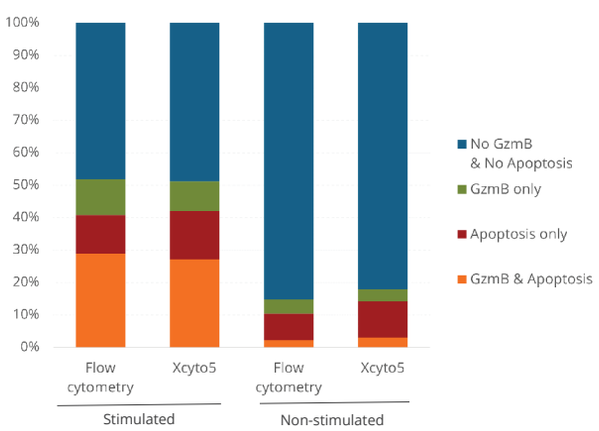

Figure 3. Comparison of data obtained by flow cytometry and Xcyto®5 image cytometer. The data represents the co-encapsulation of effector and target cells in double-emulsion droplets, assessing effector cell-mediated cytotoxicity by monitoring the release of GzmB and the induction of apoptosis.

References

1. Revealing granzyme B secretion and cell killings dynamics in a single-cell format https://samplix.com/files/samplix/downloads/AppNotes/GranzymeB-ver.01_SSX-000649-AN.pdf

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