APPLICATION NOTE

Samplix

Identifying highly potent TNF-α-secreting T cells using the Xdrop® single-cell format

Mette Juul Jacobsen, Christian Hussing, Rikke Stick Højmark, Thomas Mørch Frøsig, Peter Mouritzen, and Marie Just Mikkelsen
Samplix ApS, Birkerød, Denmark

Summary

- Bulk functional assays of immune cells miss highly potent cells within the population.
- The Xdrop droplet-based assay reveals individual highly potent TNF-α-secreting T cells in blood samples in 7 hours from start to finish.

Introduction

A single-cell view of immune cell activities, such as cytokine secretion, is critical to cell therapy research. However, most common secretion analyses use a bulk workflow, where cross-talk between secreting and non-secreting cells creates a readout bias (Figure 1, lower workflow).

Samplix has developed Xdrop and the Xdrop DE50 Cartridge to encapsulate living mammalian cells in highly stable double-emulsion droplets (DE50 droplets) for incubation, flow cytometry, and sorting. The workflows provide the required single-cell view of activity levels.

Here, we use the Xdrop workflow for TNF-α secretion analysis (Figure 1, upper workflow), identifying and isolating highly potent T cells that would be missed in a bulk assay. What's more, the workflow does not require an overnight incubation, allowing completion within a working day.

TNF-α secretion assay

Human PBMCs (peripheral blood mononuclear cells) from a healthy donor were used for a TNF-α secretion assay based on the Xdrop workflow and a bulk assay.

The PBMCs were labeled with TNF-α capture reagents according to the Miltenyi Biotec® TNF-α Secretion Assay protocol. Then, the population was split into four groups. The cells of one group were encapsulated in DE50 droplets together with TNF-α-PE antibody and cell stimulation cocktail (PMA/Ionomycin). The cells of the second group were also encapsulated with TNF-α-PE antibody but without cell stimulation cocktail. The third and fourth groups were non-encapsulated cells respectively with or without cell stimulation cocktail.

All cells were then incubated (in droplets or in bulk) at 37°C in 5% CO_2 for 1, 2, or 4 hours. TNF-α-PE antibody was added to the bulk groups after incubation and breakage of the droplets.

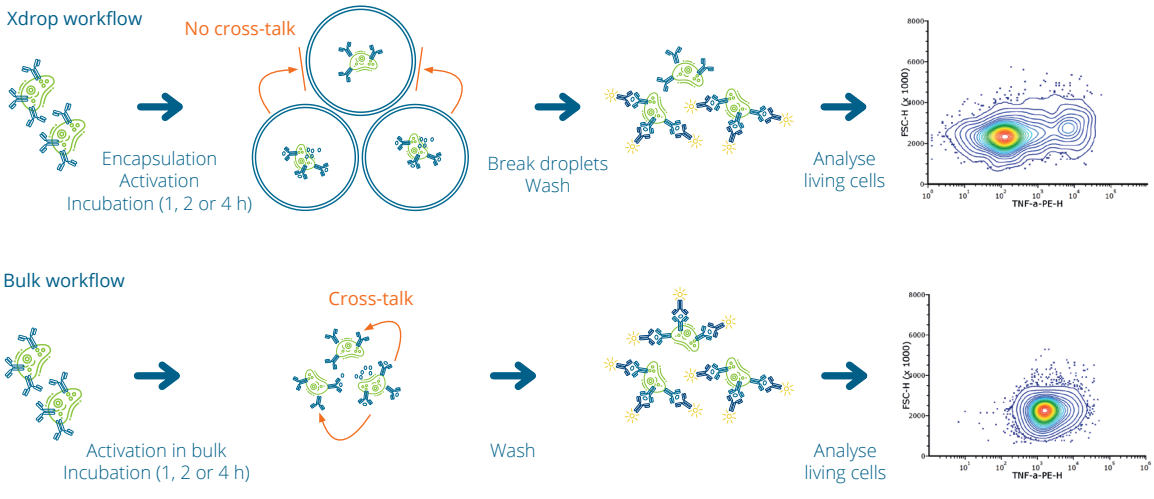

Figure 1. The Xdrop workflow for a single-cell TNF-α secretion assay with 1, 2, or 4 hours of incubation (top) and the equivalent bulk assay with the same incubation times (bottom). The whole Xdrop workflow takes 5 to 7 hours depending on the incubation time.
Set up: 1 h 20 min. Droplet generation: 8 min. Incubation: 1–4 h. Droplet breaking and staining: 1 h. Analysis on flow cytometer: 5 min/per sample. While this version of the bulk workflow takes the same amount of time, it does not deliver equivalent results.

The cells from the first two groups were then released from the droplets and all cells were washed in 0.5% BSA in dPBS, stained with live/dead-FITC stain and CD3-PerCP, and analyzed using a BD Accuri™ flow cytometer. The T cells in the samples were specifically detected via gating based on the CD3+-PerCP signal.

Figure 1 shows the Xdrop-based and bulk workflows for the activated cells. Figure 2 shows the TNF-α secretion profiles of the T cells from the blood samples.

Highly active TNF-α-secreting T cells are detected after 2 hours of incubation in droplets

After 1 hour of incubation, the TNF-α secretion profiles for the activated T cells from the bulk and Xdrop groups looked similar to each other (Figure 2 top).

After 2 hours of incubation with (activated) or without (non-activated) cell stimulation cocktail, the profile for the activated T cells from the two workflows were distinctly different, with a subpopulation of highly potent cells clearly present in the Xdrop group (Figure 2 middle). After 4 hours, this difference was even more pronounced (Figure 2 bottom). At no point is such a subpopulation of highly potent T cells discernable from the bulk activated profile.

What's more, the peak TNF-a signal for the bulk activated group increases significantly over the four hours, while the peak value for the Xdrop activated group only increases by a relatively small amount.

These results indicate that there is cross-talk between the cells in the bulk activated group, and that only a minor fraction of the cells in the bulk group are activated, but they present with a highly increased signal.

Conclusion

The Xdrop workflow using the Xdrop DE50 Cartridge allows for precise TNF-α secretion analysis in a single-cell format for human T cells in a population of PBMCs within an 8-hour working day. The incubation time is just 2 to 4 hours. Overnight incubation is not necessary. The results clearly show how significant a population of highly potent secretors can be hidden in the results of a bulk assay.

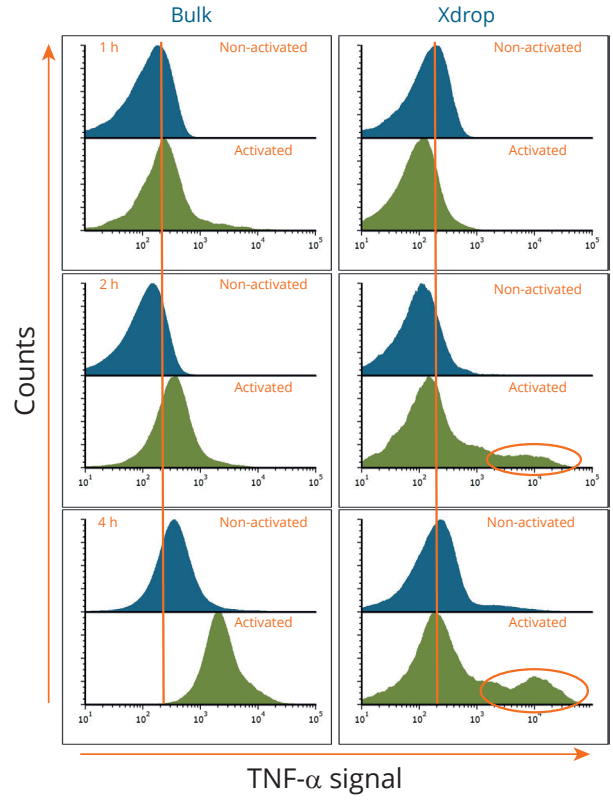

Figure 2. The TNF-α secretion profiles of the T cells from the blood samples. The cells were processed in four groups: activated and non-activated bulk groups; and activated and non-activated Xdrop groups (cells encapsulated in DE50 droplets). Top: The results after 1 hour of incubation with (activated) or without (non-activated) cell stimulation cocktail. Middle: The results after 2 hours of incubation. Note the circled area of the profile, indicating highly potent T cells. Bottom: The results after 4 hours of incubation. Note the circled area is larger and the peak for the bulk activated group is significantly different.

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