CENTRIFUGE-LESS MINIATURIZED IMMUNOSTAINING OF SUSPENSION CELLS FOR FLOW CYTOMETRY ANALYSIS USING THE DROPARRAY™ SYSTEM

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PURPOSE:

The preparation of samples for flow cytometry commonly involves staining of cells with fluorescent markers for the detection of a cell population or a cell phenotype. For over half a century this standard preparation has required the need to centrifuge and pack cells multiple times at 500xg to washing the excess fluorescent markers unbound to cells. While largely accepted in the community, such process is inherently stressful and prone to change biology of the cells. We present a new convenient methodology to wash suspension cells based on unique laminar flow properties of DropArray plate technologies DA-Cell. This technology offers a maximal retention of millions of suspension cells without the need to pack and centrifuge cells. Here, we compare DA-Cell and a conventional centrifugation method in the processing of PBMC samples for FACS analysis.

METHOD:

PBMC samples were subjected to a conventional staining preparation with Annexin V/Propidium Iodide or regulatory T cell panel in 2ml FACS tube or U bottom microtiter plate. Preparation of the same sample on DA-Cell followed equivalent antibody ratio and cell amounts as classical preparation using 96 drop based plate format. For removal of excess unbound antibody, classical sample preparation used 4X centrifugation based wash while DA-Cell sample preparation involved 7 automated cycles of aspiration/dispense with DA-Cell washer. Samples were analyzed on BD FACSSymphony™.

Figure 1: (A) Comparison of centrifuge vs DA-Cell wash method. (B) Droplet on DA-Cell plate (C) a DA-Cell µwell close up (D) DA-Cell washer close up.

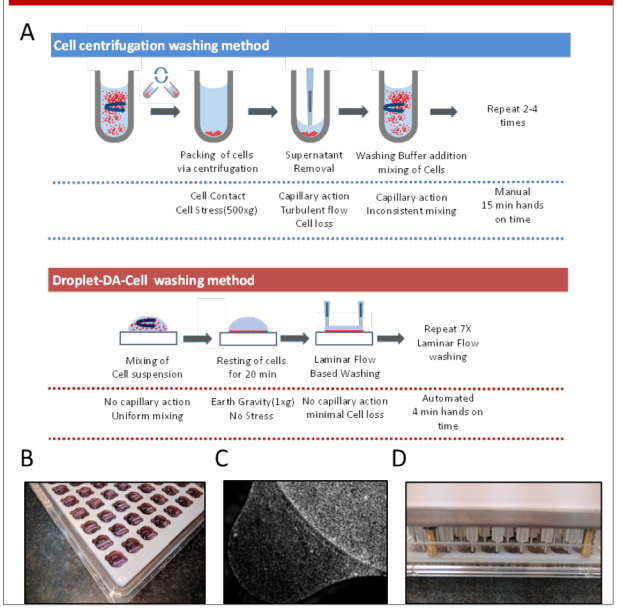


Figure 2: (A) Comparison of centrifuge vs DA-Cell wash method on cell viability. (B) Comparison of centrifuge vs DA-Cell wash method on protein expression and Ab binding.

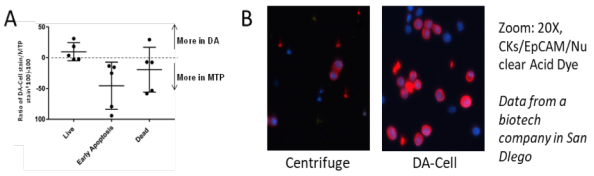


Figure 3: Comparison of 21-color assay between DA-Cell and Centrifuge wash method. DA-Cell provides effective and accurate segregation compared to those on the MTP plate.

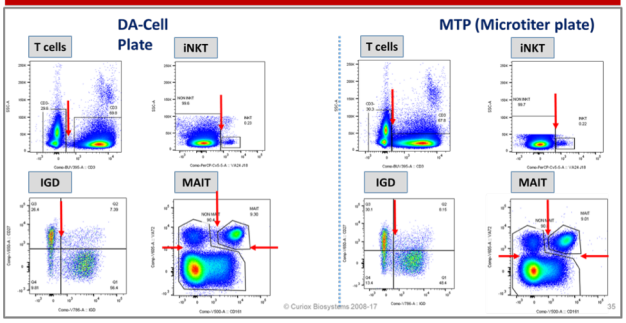
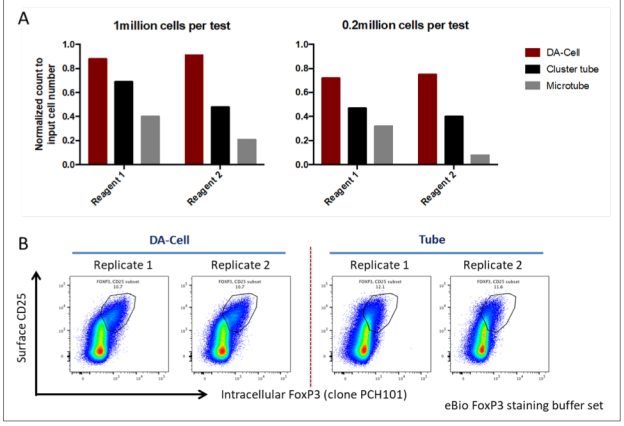


Figure 4: (A) DA-Cell wash has superior cell retention even with multi-step intracellular staining protocol such as regulatory T cell staining. High cell retention is consistent across starting cell densities or FoxP3 staining reagents from different manufacturers, when comparing DA-Cell washing against centrifugation in either 1.5mL microtubes or cluster tubes optimized in-house. (B) DA-Cell wash provides clear and distinct separation of Treg cells due to low background and high staining index of FoxP3 antibody binding, whereas centrifugated cells had much lower signal-to-noise ratio in FoxP3 staining.



RESULTS & CONCLUSION:

Results presented shows that DA-Cell technology delivers:

- **Improved viability of cells and cellular protein expression (Fig 2),**
- **Improved cell retention and avoidance of non-uniform cell loss (Fig 3),**
- **Clear and distinct identification of regulatory T cells with consistently high cell retention (Fig 4)**

With minimal hands on time, DA-Cells enables researcher to explore conveniently true biology of cells unaltered by stresses of centrifugation and compaction. The benefits of DA-Cell centrifuge-less cell processing has been successfully demonstrated in **flow cytometry**, **imaging** and **CyTOF** applications.