DEPLETION OF DEAD CELLS FROM PRIMARY TISSUE IN 6 MINUTES

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Abstract

Tissue specific research often requires mechanical and/or enzymatic digestion to isolate and study certain cell types. The digestion can be a harsh process that results in a significant number of dead cells in the final single cell suspension. Subsequent analysis by flow cytometry is difficult to interpret due to non-specific binding of antibodies to dead cells and dead cell auto-fluorescence. Factors released by dead cells can also interfere with downstream assays, complicating the study of many primary tissues.

During apoptosis, the cell membrane loses its energy-dependent phospholipid asymmetry, resulting in exposure of negatively charged phospholipids on the cell surface. Relocation of phosphatidylserine (PS) to the outer leaflet of the cell membrane is a well-established marker of apoptosis. We have developed a rapid method (EasySep™) to remove dead cells from primary tissue samples. Briefly, immunomagnetic depletion of dead cells is achieved by targeting exposed PS with Annexin V (AnxV) along with an antibody complex that links Annexin V to magnetic particles. The cell suspension is then placed in an EasySep™ magnet and live cells are poured or pipetted off and ready for use in as little as 6 minutes.

Performance of this kit was examined for various mouse and human tissue types. Using this method, we were able to improve viability of a single-cell suspension of mouse lungs digested with collagenase/hyaluronidase from an initial viability of 40.6 ± 12.1% AnxV$^-$/PI$^-$ to 70.7 ± 11.2% AnxV$^-$/PI$^-$. From 1x10^8 total start cells, 1.50 ± 0.68 x10^7 live cells were recovered (n = 11). From human polymorphonuclear leukocytes (PMN) cultured overnight, viability was improved from 22.5 ± 9.7% AnxV$^-$/PI$^-$ to 69.7 ± 12.5% AnxV$^-$/PI$^-$ with recovery of 1.19 ± 0.53 x10^7 live cells from 1x10^8 total start cells (n = 11). This equates to removal of 88.2 ± 8.2% and 92.7± 5.5% dead cells respectively. Since live cells are untouched, subsequent isolation of desired cell types can be performed, resulting in a more viable population of cells for downstream applications.

Methods

Preparation of Starting Cell Suspension

Polymorphonuclear Leukocytes (PMN): PMN were prepared by ficolling whole blood, removing the mononuclear cell layer and lysing the pellet with ammonium chloride. PMNs were then cultured at 37°C overnight in RPMI + 10% FBS.
Mouse Lungs: To generate a single cell suspension, lungs from wildtype C57BL/6 mice were first cut into small pieces, then enzymatically digested with collagenase/hyaluronidase and DNase I in RPMI at 37°C for 20 minuntes. This suspension was passed through a 70 µm strainer.
Mouse Spleens: To create a single cell suspension, spleens were harvested from wildtype C57BL/6 mice and passed through a 70 µm nylon mesh cell strainer. The cell suspension was left at 4°C for 24 - 48 hours.

EasySep™ Dead Cell Depletion Strategy

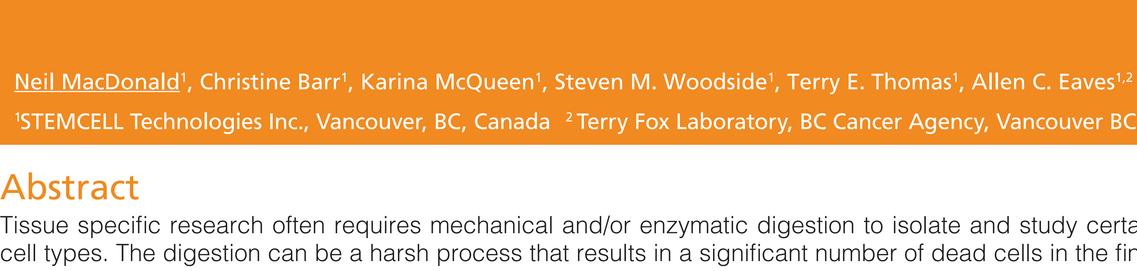

FIGURE 1. Dead Cells Were Removed with EasySep™ Dead Cell Removal (Annexin V) Kit (Catalog #17899)
Dead cells (Annexin V$^+$) were targeted for depletion using Annexin V, antibody complexes and magnetic particles and then placed in an EasySep™ magnet. Labeled dead cells were retained in the magnet while untouched live cells were poured or pipetted off.

Assessment of Viability

Viabilities for start populations and dead cell depleted samples were assessed by flow cytometry using fluorochrome conjugated Annexin V in the presence of propidium iodide. Live cells are Annexin V$^-$/PI$^-$.

Results

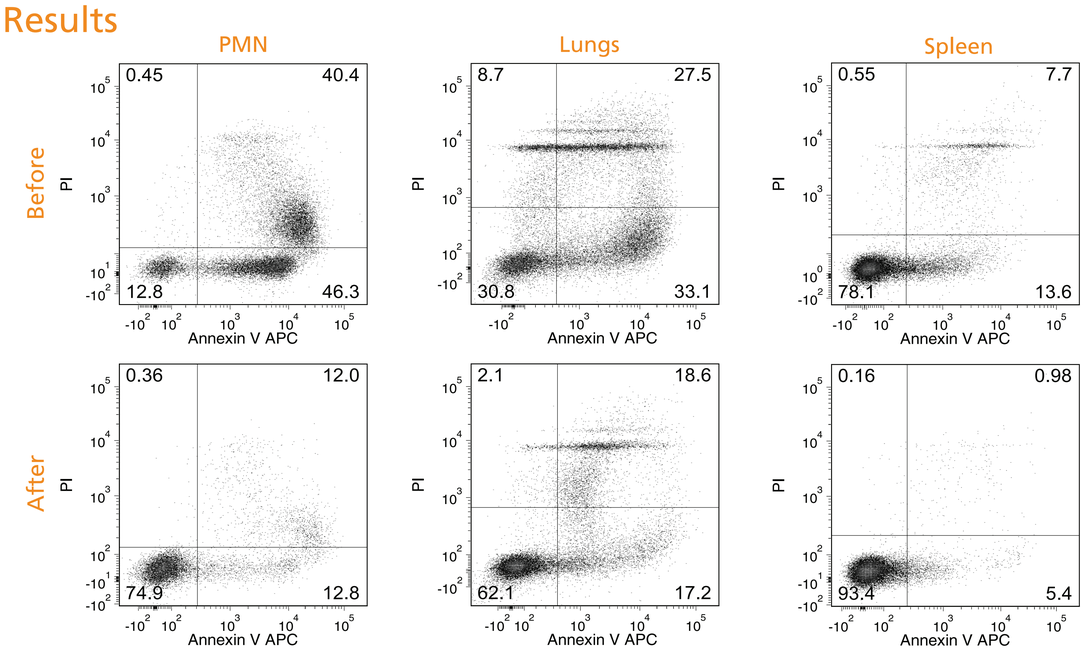

FIGURE 2. Representative Flow Cytometry Data Before and After EasySep™ Depletion of Dead Cells From Human PMN, Mouse Lung or Mouse Spleen

Sample Type	n	Start % Viability	% Viability of Depleted Fraction	Live Cells Recovered per 1 x 10^8 Total Start Cells (x10^7 Cells)	% Dead Cells Removed
Human PMN	11	22.5 ± 9.7	69.7 ± 12.5	1.19 ± 0.53	92.7 ± 5.5
Mouse Lung	11	40.6 ± 12.1	70.7 ± 11.2	1.50 ± 0.68	88.2 ± 8.2
Mouse Splenocytes	12	64.5 ± 10.6	79.8 ± 11.4	3.76 ± 0.98	75.2 ± 11.8

*All Values are Mean ± SD

TABLE 1. Viability Before and After EasySep™ Dead Cell Depletion, Number of Live Cells Recovered, and Percentage of Dead Cells Removed
Data is shown for three different sample types: human PMN, mouse lung, and mouse splenocytes. Viability is shown as percent of cells that are Annexin V$^-$/PI$^-$ by flow cytometry. Average cell yield is presented as the number of live cells (x10^7) per 1 x 10^8 total start cells. Percent of dead cells removed is shown as mean ± SD.

FIGURE 3. Over 84% of Dead Cells Were Removed Following Depletion with EasySep™ Dead Cell Removal (Annexin V) Kit
The above graphs show the number of dead cells after dead cell depletion obtained from 1 x 10^8 total start cells in PMN, mouse lung and mouse splenocytes.

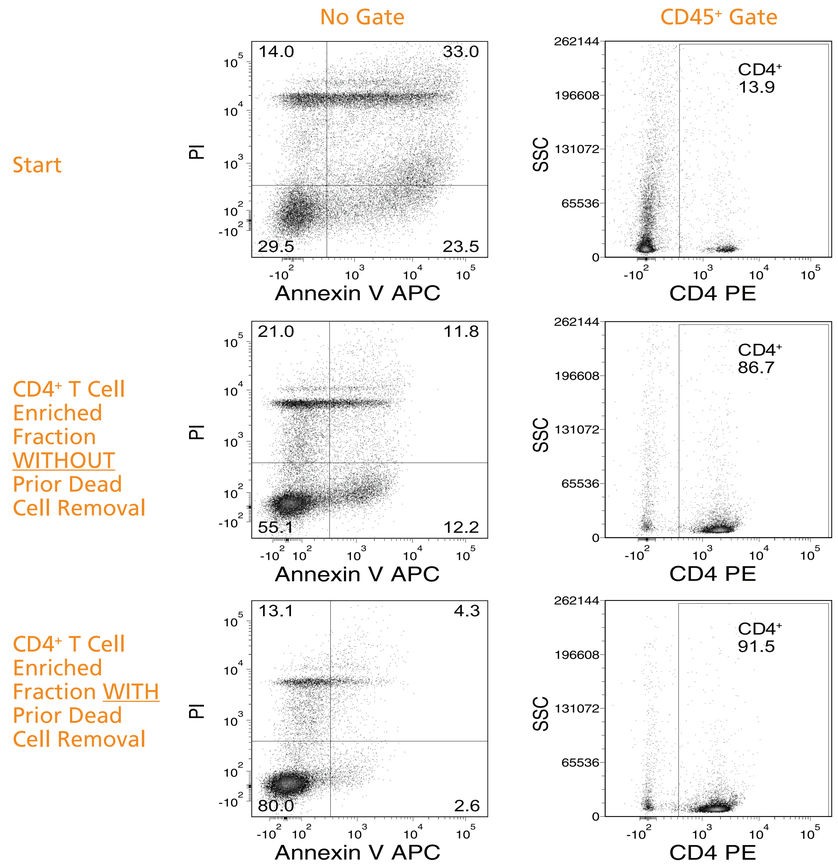

FIGURE 4. Depletion of Dead Cells with EasySep™ Dead Cell Removal (Annexin V) Kit Prior to Subsequent Cell Isolation Results in Higher Cell Viability and Improved Purity of Isolated Cells
In this example, EasySep™ CD4+ T cell enrichment (using Catalog #19852) was performed on a single-cell suspension from mouse lungs with or without dead cell depletion using the EasySep™ Dead Cell Removal (Annexin V) Kit prior to isolation.

Summary

- Fast, easy-to-use and column-free immunomagnetic depletion of dead cells in as little as 6 minutes
- Compatible across tissue types and species
- Untouched live cells are ready to use in downstream applications or subsequent separations
- Improved viability of cells obtained in subsequent separations