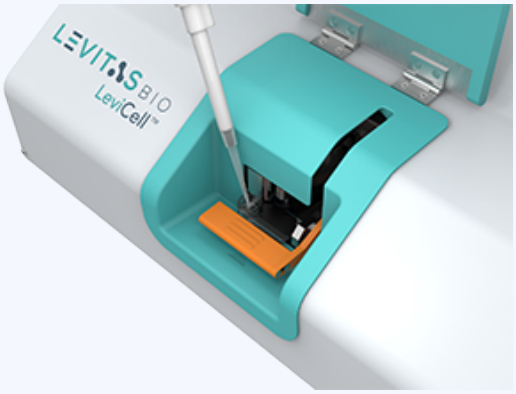
LEVITAS BIO

LeviCell™ System Enables Macrophage Research

Introduction

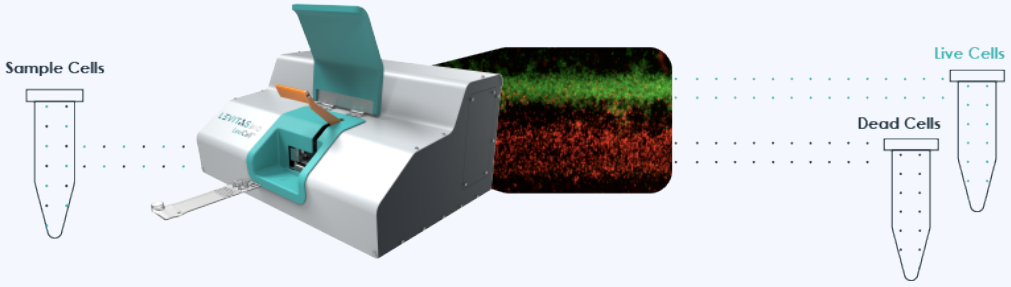
As scientists and researchers working with macrophages in vitro know all too well, the promise of new and powerful insights in science that these key cell types offer come at a price of time, frustration, and challenges in reproducing results. Mature differentiated macrophages are present in very low numbers in various human tissues, while the various downstream assays need a sufficient quantity of cells to be separated at a certain level of purity. Macrophages cannot proliferate in vitro – they need to be isolated and differentiated with specific media – processes that take valuable time. Adding to the challenge of working with macrophages, they are notoriously sensitive to handling using traditional methods and techniques.



The LeviCell is proven to successfully sort live macrophages with class-leading yields and viabilities – without affecting activation state – for accurate downstream analysis in 20 touch-free, label-free, frustration-free minutes. Furthermore, result reproducibility is maximized because the entire sorting and separation process occurs in the completely closed and sterile environment of the LeviCell and involves only 3 simple manual steps (instead of the complex 13-16 step processes of current methods).

Method

Levitas Bio has developed an innovative levitation technology platform that uses less than 1 psi of pressure to enable a completely touch-free, label-free, three-step macrophage sorting process that takes only 20 minutes to complete.



1 Sample Introduction
Sample pipetted into the LeviCell cartridge.

2 Automated Label-Free Sorting
Magnetic force drives levitation based on cells' physical properties.

3 Sample Collection
Levitated cells transition into separate collection ports for removal.

Live macrophage populations levitate much higher than dead cells and debris, allowing for clear separation and collection by the LeviCell, as shown. Please note that the fluorescent dyes used in the provided image were used solely to highlight the separation process and are not required by the LeviCell.

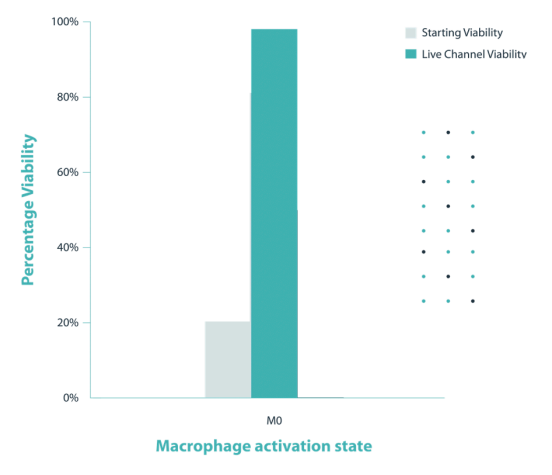
Results

LeviCell enriches for the live macrophage cells

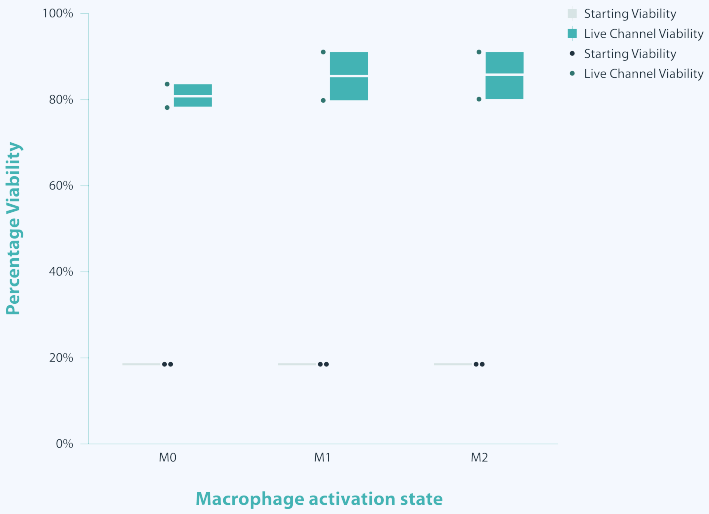
The RAW264.7 mouse macrophage cell line was used to determine whether the LeviCell was gentle on fragile cells such as macrophages.

Cells were harvested from culture (RAW264.7 cells are cultured in DMEM buffered with 10% FBS) and their viability determined by Trypan blue staining. RAW264.7 macrophages were then washed in levitation agent and immediately introduced into the LeviCell.

The starting viability was measured at 21% (grey bar). In the LeviCell, live cells levitated to the top channel while the dead cells could be found levitating lower. The LeviCell sort (green bar) enriched the Live channel to a viability of over 90%! This demonstrates that not only is the LeviCell able to enrich live cells, but it is also gentle on the cells, resulting in very little damage to the cells during the sort.



LeviCell maintains macrophages' polarization state

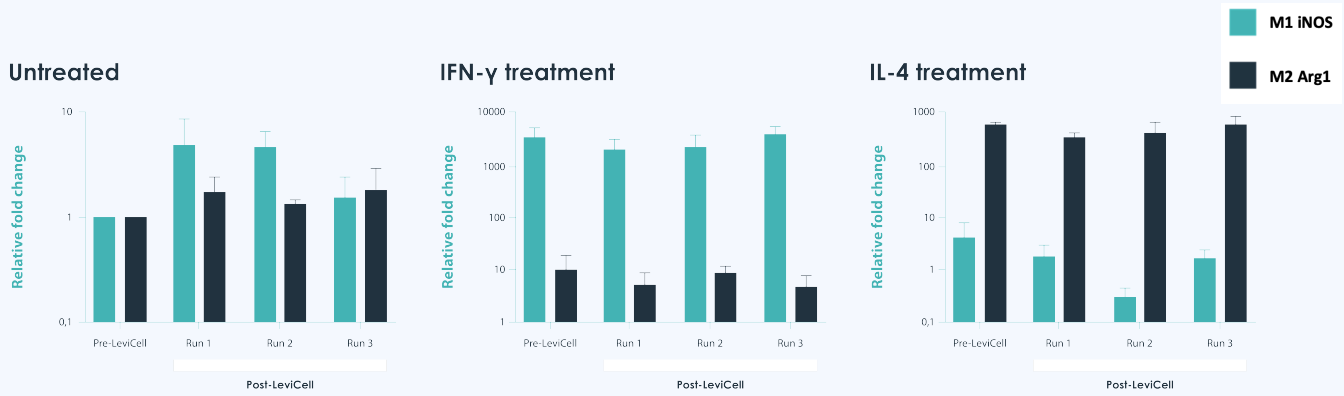


After demonstrating that the LeviCell could enrich for live macrophage populations, we tested the LeviCell's ability to sort macrophages without affecting their activation states.

Macrophages can be polarized into two main states: M1 and M2. M1 macrophages are pro-inflammatory and characterized by the production of enzymes such as iNOS and cytokines like IL-12. M2 macrophages are anti-inflammatory and express intermediates such as Arg1 and produce IL-10.

To generate M1 and M2 macrophages, J774 mouse macrophage cell line (cultured in DMEM buffered with 10% FBS) was treated with IFN-γ (50ng/mL) and IL-4 (20ng/mL) respectively for 24h. Untreated macrophages (M0) were kept as control. The starting viability of the J774 cells going into the LeviCell was 20% for each polarization state.

We observed an enrichment to 88-100% viability post-LeviCell sort. We took a step further and performed qRT-PCR, looking at the expression of genes indicative of the M1 (iNOS) and M2 (Arg1) states in these cells to show that their activation states were not compromised by the LeviCell sort.



No differences in gene expression were observed between the input and output cells for all the activation states. This shows that the LeviCell sort is gentle enough to sort macrophages without affecting their polarization states, thus working as a powerful tool for studying the functionality of these cells.

Conclusion

Various sorting methods and techniques for sensitive cell types such as macrophages typically take a researcher with a high level of expertise several hours to conduct. Traditionally, this involves a tedious, manual workflow that often results in low yield, damaged cells, and low viability. The LeviCell's 3-step, hands- and label-free platform enables the enrichment of fragile cell types while maintaining their gene expression and original sample representation for accurate downstream analysis. Faster, easier, fewer losses, high viability, no debris – all in 20 minutes.

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