Magnetic levitation-based viable cell enrichment minimizes isolation-induced inflammatory response signaling in mammalian immune cells

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ABSTRACT

Isolation of single cells from tissue with minimal transcriptional profile changes is critical to elucidating the link between gene expression and disease. Widely used enrichment methodologies can exert undue stress on cells, altering their native transcriptomic profiles. This method-related dysregulation of genes and/or pathways often masks more biologically-relevant gene expression profiles and can be misinterpreted as a consequence of disease. Certain cell types such as brain resident microglia and the immune fraction of Dissociated Tumor Cells (DTC) are highly sensitive to stress or other stimuli, which triggers increased inflammatory responses due to cell handling. Here, we report on a novel, gentle and quick, label-free enrichment procedure that harnesses magnetic levitation to efficiently isolate a higher percentage of microglial cells from fresh rodent brains without inflicting significant cell stress. Magnetic levitation-enriched samples yield more homeostatic microglia and fewer proliferating and inflammatory microglia as compared to cells isolated by flow cytometry. Expression of genes associated with inflammatory pathways were increased (2-6x fold) in flow-processed cells compared to the magnetically-levitated cells, suggesting that flow cytometry workflows significantly alter the transcriptomic profile of these cells. Similar results were observed in the immune cell component of DTC samples, where certain inflammatory pathways were found to be upregulated when flow cytometry was used to enrich for viable cells compared to magnetic levitation. These changes in gene expression may be misinterpreted as disease-related phenotypes. In addition to significantly lowering expression of inflammation-related genes, magnetic levitation-enriched cells had higher quality sequencing metrics after scRNA-seq. Median genes per cell, fraction reads per cell, and median Unique Molecular Identifier (UMI) per cell were all higher in magnetically-levitated samples. A concomitant decrease in mitochondrial content per cell was also observed.

In conclusion, using two different tissue types, we unequivocally demonstrate the effectiveness of Levitation Technology to isolate and enrich cells in their native transcriptomic state, enabling researchers to study disease-related pathways and/or targets that reflect their true biology, not stress-induced activity.

LEVITATION TECHNOLOGY

LevitasBio has developed the LeviCell™ platform, a powerful novel technology for cell separation and characterization that utilizes magnetic fields to levitate cells. Unlike other methods, cell separation via magnetic levitation does not require dyes, antibodies, specific markers, or magnetic beads, and the cells are not modified or perturbed in any fashion. However, magnetic levitation also does not preclude the use of these tools to further enhance separation of cells. In its simplest form, magnetic levitation requires only the addition of an inert paramagnetic compound to the media in which the cells are suspended. The cells are subsequently introduced into a single use cartridge within an externally applied magnetic field which causes the cells to levitate in solution. The intrinsic properties of the cells, including density and magnetic susceptibility, determine the height to which the cells levitate in the cartridge channel. Since different cell types often have different properties they levitate to different heights, enabling their separation. Continuous channel imaging allows for sample analysis and characterization with the benefit of real-time control over the cell collection. The simplicity of magnetic levitation enables cells to be treated gently without use of high pressure or other perturbations that commonly lead to increased cellular stress responses, specific cell type activation, or even cell death. The direct and gentle collection flow path leads to high yields of live cells even with very low input cell numbers.

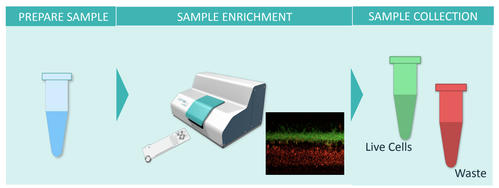

Figure 1. Broad view schematic of the LeviCell workflow. The LeviCell workflow involves three basic steps, namely sample preparation (simple addition of inert paramagnetic medium to sample), enrichment and collection of viable cells.

BASIS OF LEVITATION

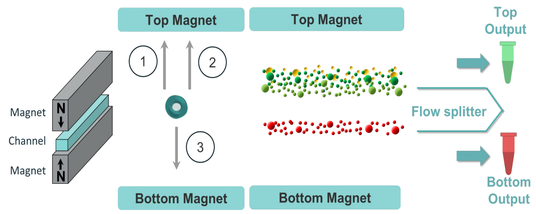

Figure 2. The basis of levitation within the LeviCell workflow. The separation channel containing cells in a paramagnetic medium is placed between two magnets. Within the cross-section of the separation channel, cells reach their final levitation height determined by a combination of buoyancy (1), magnetic forces created within the paramagnetic fluid (2), and gravity (3). This view illustrates different cell populations at different levitation heights. Live cells (shown in yellow and green) will levitate high and according to their density or magnetic properties. Dead cells (red) will levitate lower in the channel since their membranes are permeable to the paramagnetic media. After levitation, the separated cells are harvested into the output ports, where they can be collected for downstream use.

ENRICHMENT VIA LEVITATION RETAINS ALL RESIDENT BRAIN CELL TYPES, WITH HIGHER NUMBER OF HOMEOSTATIC MICROGLIA

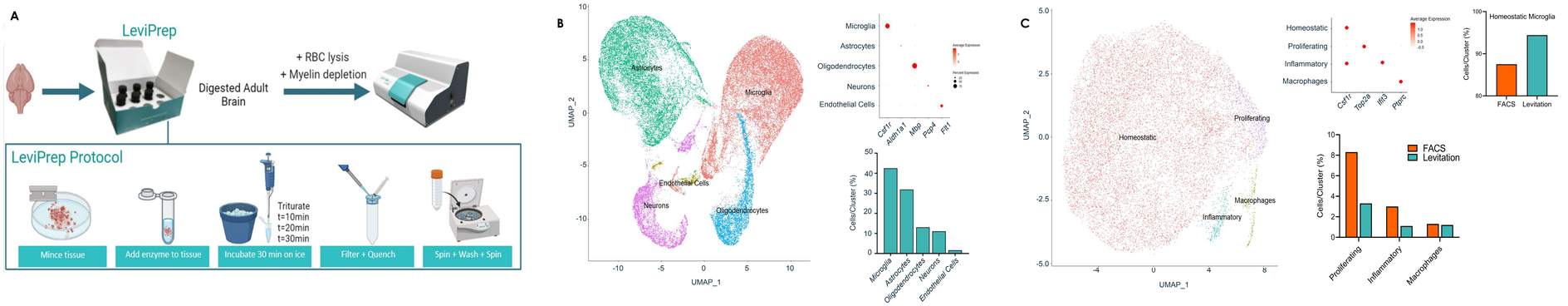

Figure 3. LeviPrep-LeviCell workflow efficiently enriches all resident brain cell types, with higher proportion of homeostatic microglial cells. (A) A workflow combining LeviPrep™ tissue dissociation with magnetic levitation was designed to isolate and enrich all the different brain-resident cell types. The LeviPrep™ workflow includes enzymatic tissue dissociation, trituration, filtration, centrifugation, Red Blood Cells (RBCs) and myelin removal before introducing them to the LeviCell™ system. (B) Using cell subtype specific markers corresponding to neurons (Pcp4), microglia (Csf1r), astrocytes (Aldh1a1), oligodendrocytes (Mbp) and endothelial cells (Flt1), we demonstrate the presence of all resident brain cell subtypes in varying proportions. (C) Using specific markers, we identified different microglial subtypes based on their cell state. The LeviPrep followed by the LeviCell workflow enriches homeostatic microglia whereas, flow cytometry sorting led to a higher proportion of activated microglia.

FLOW CYTOMETRY PRODUCES UP TO 6X HIGHER EXPRESSION OF ACTIVATION MARKERS AS COMPARED TO LEVITATION

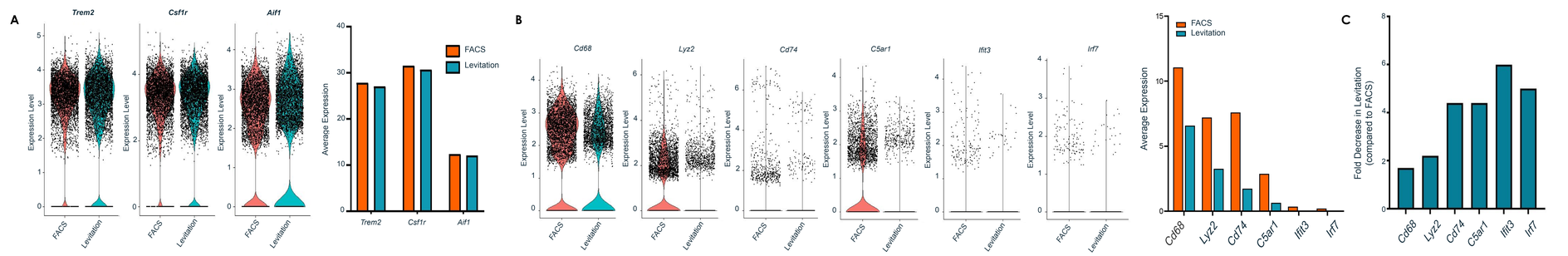

Figure 4. Enrichment of microglial cells by flow cytometry leads to activation of various stress pathways, not observed in microglial cells enriched via Levitation. (A) Based on the expression of various homeostatic and activated microglial markers, we observed that the levitation workflow enriches microglia with 2-6X less activation-inducing stress (C) and altering transcriptional signature. (B) This is depicted by lower expression levels of activated gene markers of various known stress pathways such as the Lysosomal pathway genes (Cd68, Lyz2), antigen presenting gene (Cd74), Complement pathway (C5ar1) and Interferon response genes (Ifit3, Irf7) as compared to the FACS processed microglia.

CELL SORTING INDUCES STRESS AND ACTIVATES IMMUNE RESPONSE PATHWAYS, HIDING TRUE CELL BIOLOGY

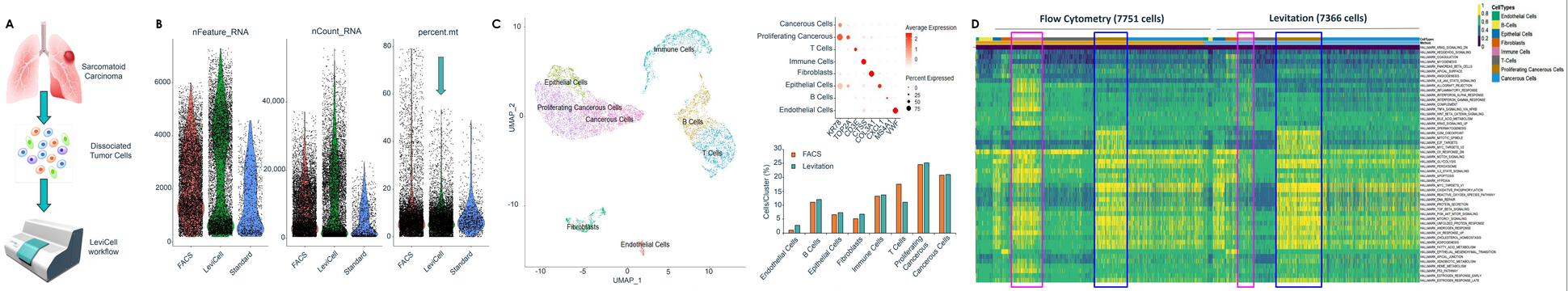

Figure 5. Enrichment via Levitation enables us to study inflammatory pathways without inducing additional activation in human Dissociated Tumor Cells (DTCs). (A) We subjected human DTCs through the Levitation workflow to obtain viable cells. (B) The percent mitochondrial content (Percent.Mt) was significantly lower (blue arrow) in cells enriched with the Levitation workflow as compared to the flow cytometry enrichment method. The number of transcripts per cell (nCount_RNA) and the number of genes detected per cell (nFeature_RNA) was also higher in cells enriched with the Levitation workflow. (C) Using cell type specific markers, we annotated and obtained eight different cell types namely, cancerous cells (KRT8), proliferating cancerous cells (TOP2A), B-cells (MS4A1), T-cells (CD3E), immune cells (CTSS), fibroblasts (CCL3A1), epithelial cells (CXCL1) and endothelial cells (VWF). Upon quantification, we observed similar proportions of cells per clusters after the enrichment via the Levitation workflow (7366 cells) as compared to the flow cytometry (7751 cells) enrichment. (D) Using the Hallmark Gene Set in Gene Set Enrichment Analysis (GSEA), we compared activation of various pathways in cells processed with flow cytometry (on the left) or with levitation (on the right). Immune cells (highlighted in pink boxes) show more activation when processed with flow cytometry. In contrast, other cell types like cancerous cells (highlighted in blue boxes) showed similar expression of most of the studied pathways, irrespective of the enrichment method.

CONCLUSION

Levitation Technology :

- Efficiently enriches and maintains native representative populations from diverse tissue types.
- Retains homeostatic states of sensitive cell types like microglia in the brain.
- Avoids sample induced activation and gene expression changes seen with traditional cell sorting methods.
- Allows effective characterization of tumor microenvironment to identify true biological activity.
- Redefines tumor infiltrating lymphocytes basal states and provides access to potential new druggable targets/pathways.

REFERENCES

Durmus, N.G., et al., "Magnetic levitation of single cells", *Proceedings of the National Academy of Sciences*, 2015, 112(28):E3661-8

Hammond, T.R., et al., "Single-Cell RNA sequencing of microglial throughout the mouse lifespan and in the injured brain reveals complex cell-state changes", *Immunity*, 2019, 50(1):253-271.