HIV-INFECTED CD4 T CELL ISOLATION USING A MICROFLUIDIC MAGNETIC LEVITATION SYSTEM

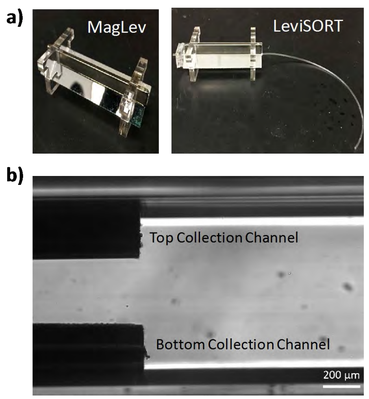
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Introduction: Strategies to identify and isolate HIV infected cells at various states of latency or transcriptional activation are urgently needed. We developed and implemented a novel microfluidic system that is able to sort untouched CD4+ T cells from HIV-infected individuals based on levitation heights within a magnetic field (*i.e.* magnetic density). We compared HIV DNA and unspliced RNA burden and immune phenotypes in cells from high and low density populations.

Methods: Untouched CD4+ T cells from ART-suppressed individuals were sorted based on magnetic density using our novel micirofluidic magnetic cell levitation and sorting system. A microfluidic chip-based platform incorporates collection channels for high-throughput isolation of cells that levitate at different heights in a biocompatible paramagnetic medium, allowing for downstream characterization of HIV burden and immune phenotype.



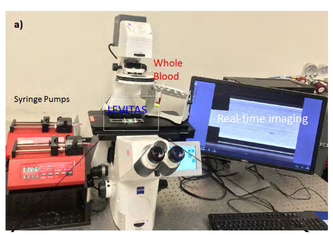


Figure 1. LeviSORT platform for high-throughput cell sorting based on magnetic levitation principles. **a)** A static magnetic levitation chip consists of PMMA, permanent neodymium magnets and tilted mirrors. **b)** LeviSORT-chip for high-throughput sample processing with the inlet and sample collection outlets for low and high density cell isolation.

Higher Levitation of *In Vitro* HIV Infected CD4+ T Cells

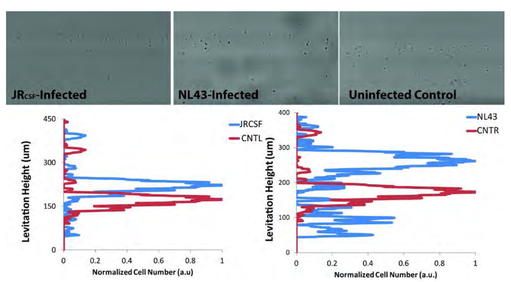
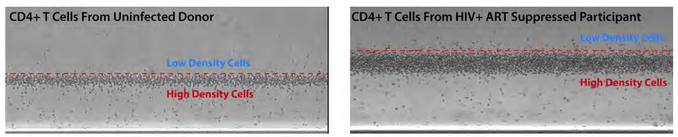


Figure 2. MagDense levitation profiles of laboratory-infected, resting CD4+ T cells compared with uninfected control cells. Both JRCSF and NL43-infected cells levitated higher than uninfected cells. The difference in levitation height of the JRCSF-infected cells was greater than that obtained with *ex vivo* stimulation of CD4+ cells.

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Levitation Heights, Density Dispersion, and Size of CD4+ T Cells From Uninfected and ART-Suppressed HIV+ Participants



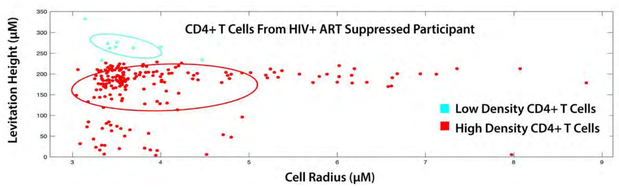


Figure 3. Increased frequency of low density CD4+ T cells in samples from HIV-infected participants on suppressive ART.

High-Throughput Isolation of Untouched CD4+ T Cells Based on Cell Levitation Height

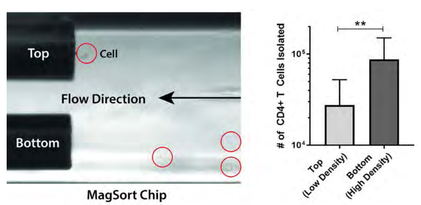


Figure 4. CD4+ T cell counts recovered in high and low density layers using initial and refined LeviSORT protocols from a single cell aliquot. The LeviSORT system was tuned to differentiate cells with different magnetic densities within untouched total CD4+ T cells obtained from ART-suppressed individuals.

CD4+ T Cells That Levitate at Different Heights Have Similar Activation and Subset Phenotypes Determined by Flow Cytometry

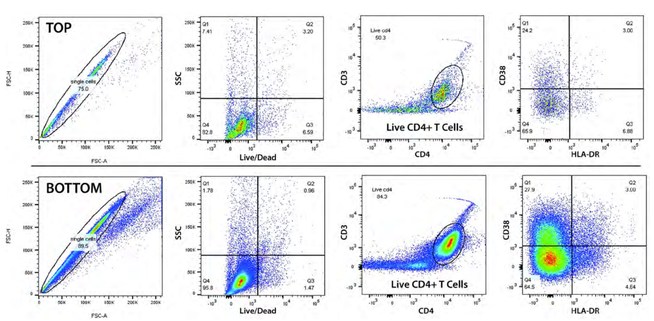


Figure 5. Flow cytometric characterization of high and low-density CD4+ T cells isolated by the LeviSORT system. CD4+ T cells based on levitation height were similar with respect to CD4+ T cell populations and activation markers in all samples tested. Similar frequencies of CD38/HLA-DR expression and CD69 and CD45RA expression (not shown) were observed.

Higher HIV DNA Levels in Lower Density CD4+ T Cells From ART Suppressed Participants

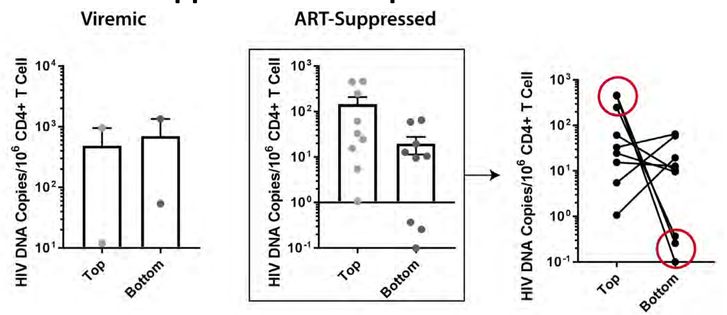


Figure 6. HIV-1 DNA levels per CD4+ T cell in high and low density levitation layers. HIV-1 DNA was almost exclusively detected in the top, low-density layer in one third of patient samples from ART-suppressed individuals.

Higher HIV RNA Levels and RNA/DNA Ratio in Higher Density Cells

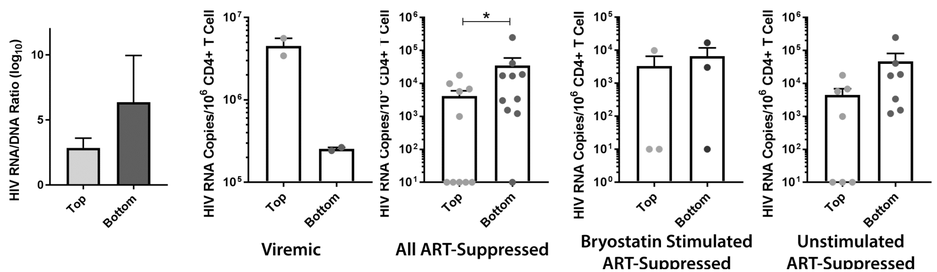


Figure 7. HIV-1 RNA levels per CD4+ T cell in high and low density levitation layers. Unlike cell-associated DNA, RNA levels were lower in the top, lower-density cell layer. This result was opposite of what was identified in viremic individuals, where lower-density cells contained very high levels of HIV RNA (in some instances more than one copy per cell).

Conclusions

- HIV infected CD4+ T cells of various transcriptionally active states have unique magnetic levitation/density characteristics
- Levitation height may be independent of surface protein marker expression, cellular activation state, and cell size
- HIV DNA was observed almost exclusively in lower density CD4+ T cells in three samples whereas HIV RNA levels and RNA/DNA ratios were higher in higher density CD4+ T Cells
- Magnetic levitation isolation has the potential to be refined for HIV reactivation, latency or eradication studies