TECHNICAL NOTE

Enhancing Enrichment by Modulating Levitation Height

Introduction to Levitation Technology

The LeviCell® platform is an exciting new technology that uses a magnetic field to separate cells based on their intrinsic physical properties. In its simplest form, levitation occurs through the use of a magnetic field in the presence of an inert paramagnetic compound (Levitation Agent) which is added to the cell suspension media. The cell suspension is loaded into the separation cartridge, where it is then exposed to a magnetic field. Cellular density, buoyancy, and magnetic susceptibility determine the height to which the cells levitate within the separation channel. Cell separation occurs when different cell types equilibrate to different levitation heights within the levitation media based on their native properties. The LeviCell systems have imaging capabilities that allow for real-time visualization of the sample while it levitates during the run. This also provides the ability to define how the sample will be divided between the fraction of interest and the waste prior to collection. The images captured allow for post-collection analysis and charaterization.

The simplicity of Levitation Technology™ enables cells to be treated gently, since there is no cellular exposure to high pressures or other perturbations that commonly lead to increased cellular stress responses, specific cell type activation, and cell death.

With no requirement for dyes, antibodies, specific markers, or magnetic beads, live cells can be enriched without the concern of process-related perturbations of their native states. If more targeted methods for cell enrichment are required, Levitation Technology can be adapted to meet user needs. In such a situation, various labeling technologies can be employed to augment the power of levitation-based cell enrichment.

Modulation of Levitation Height

Since Levitation Technology leverages the variations in the density and magnetic susceptibility of cells, any changes to these properties can alter levitation height. Examples of such cell alterations are: genetic disorders such as thalassemia[1], cell differentiation (internal data), or binding of cell surface epitopes with an antibody conjugated to a low-density, high-density, or magnetic particle. Combining these factors can be used to add further dimensions to cell separation and sample processing.

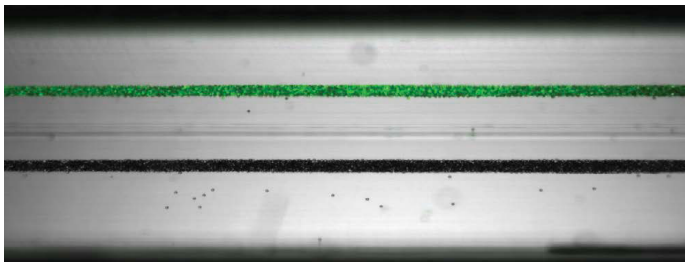

Figure 1. Effect of low- and high-density particles on levitation height. *Demonstration of the differential levitation heights of both low-density (green) and high-density (black) polymers.*

To demonstrate how density differences affect levitation, we can levitate polymer microparticles composed of materials with different densities. The denser the particle, the lower it will levitate in the magnetic field compared to a less dense particle. Thus composition and magnetic susceptibility of particles can alter the levitation height.

By leveraging this understanding of differential material density, cell-specific antigens, and magnetic particles, we are able to target subsets of application-specific cell types using Levitation Technology.

To demonstrate the ability to alter cell levitation height, we first examined the use of small dense particles that are directly conjugated to a cell surface receptor specific antibody. Jurkat Clone E6.1 (T-cell) cells and H358 (lung epithelial) cells were mixed in this experiment. The Jurkat cells were labeled with Calcein-AM to visualize the populations more effectively and use the fluorescent imaging capabilities on the LeviCell 1.0 system.

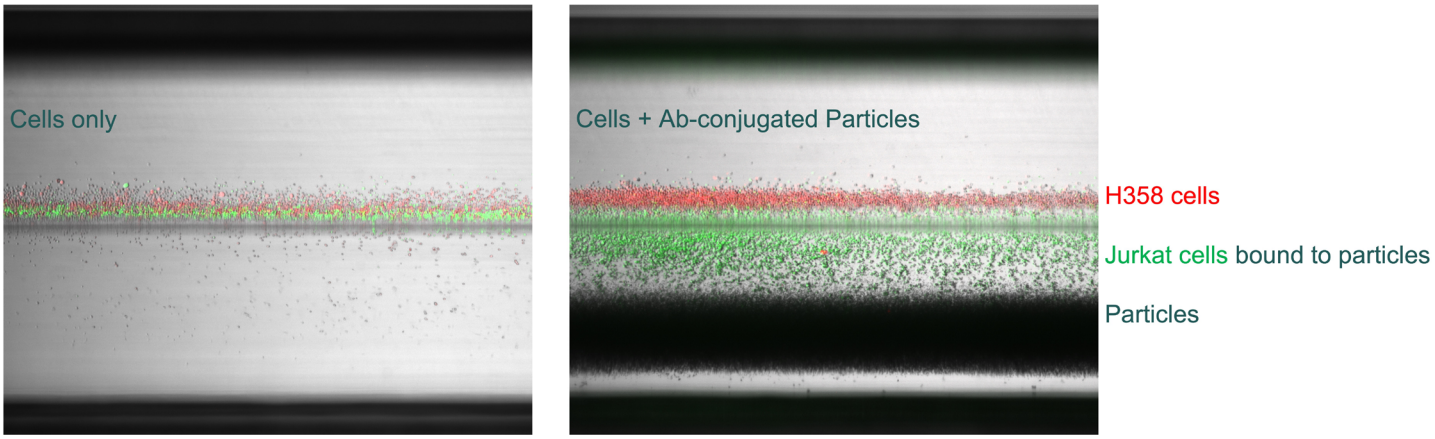

Figure 2. Separation of cell types with density particles. *In the first two images on the left, the fluorescently labeled Jurkat cells (green) are located at the bottom of the channel due to the highest concentrations of magnetic particles. As the concentration is decreased, the Jurkat cells can be observed levitating at increased heights. In the final image on the right, there are no magnetic particles present, resulting in no differences in levitation height between the two cell types.*

In contrast, H358 cells were labeled with CellTracker™ Red CMTPX Dye (Thermo Fisher Scientific PN C34552). The cells were mixed at a 50:50 ratio prior to incubating both with and without anti-human CD45 antibody-labeled higher density microparticles.

As expected, binding of the CD45 antibody was only observed with Jurkat cells, as demonstrated in Figure 2. This binding resulted in a lowering of the levitation height of the Jurkat cell population in the LeviCell 1.0 system. Due to the number of particles bound per Jurkat cell, they equilibrated to a new height that is in between the levitation heights of unbound cells and particles alone. Particles alone, which are quite dense, levitated at a much lower height in the separation channel of the LeviCell cartridge, demonstrating that the particle density is higher than the complex formed between the Jurkat cells and particles.

In another example experiment, we looked at CD45 antibody-labeled magnetic particles and their ability to alter levitation height. By varying the concentration of cellbound magnetic particles, we found measurable differences in the levitation height of CD45+ Jurkat cells. A cell mixture in a ratio of 85:15 of Calcein-AM-labeled Jurkat to CellTracker™ Red CMTPX Dye (Thermo Fisher Scientific PN C34552)- labeled H358 cells, respectively, was added to varying amounts of antibody- conjugated magnetic particles. The levitation height of the H358 cells was unchanged as compared to the no magnetic bead control sample (Figure 3, next page). At intermediate ratios of magnetic particles to input cells, the Jurkat cells levitated at different heights compared to the no bead sample. The balance of magnetic forces, the Jurkat cells own buoyancy and magnetic susceptibility within the paramagnetic medium, and the number of magnetic particles added to the cell suspension (i.e. the number of particles bound per cell) enabled the separation of the Jurkat cells from the H358 cells. This experiment demonstrated that when the concentration of magnetic particles on the cell surface was high, the Jurkat cells were pulled to the bottom of the separation channel. In contrast, when the concentration of magnetic particles on the cell surface became more limited the cells remained in view but at varying lower levitation heights. The concentration of paramagnetic Levitation Agent can also influence the spread of the Jurkat cell band in the separation channel (data not shown).

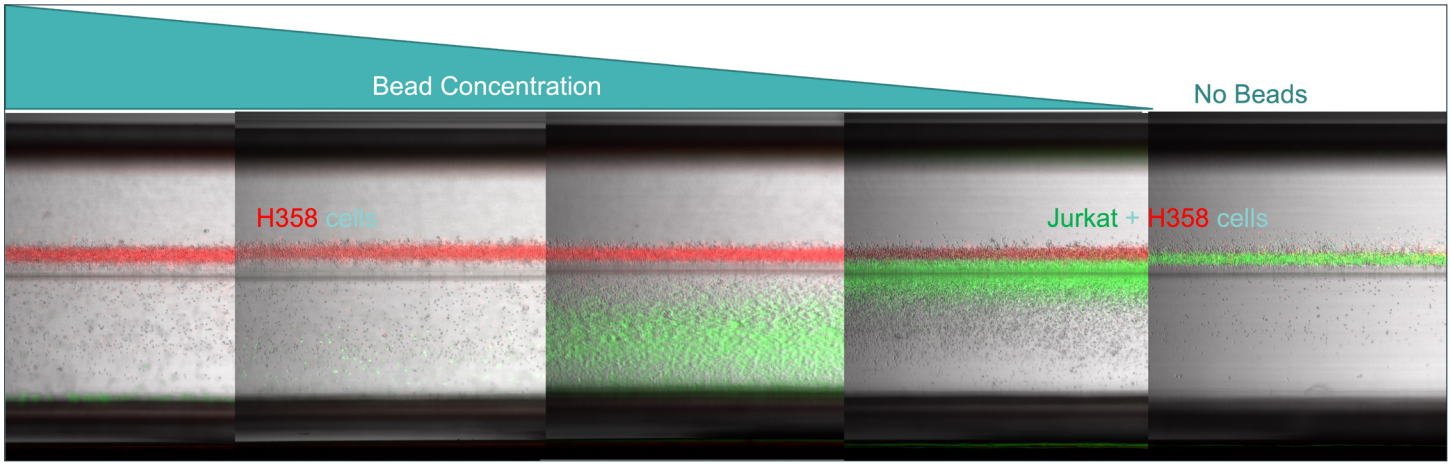

Figure 3. Inverse correlation between magnetic particle concentration and levitation height. *(Left) In their natural state, Jurkat (green) and H358 (red) cells levitate at similar heights. (Right) Separation of the two cells types was achieved with the addition of antibodyconjugated CD45 high-density polymer particles, which bound to the Jurkat cells and lowered their levitation height. CD45 high-density polymer particles, which bound to the Jurkat cells and lowered their levitation height.*

Conclusion

Identifying and enriching cells of interest from a mixture of different cell types is fundamental to the study of basic cell biology. While magnetic levitation alone can enrich live cells from dead or dying cells and can separate cells based on their inherent differences, the ability to modulate levitation height will further enhance the power of this technology. We have demonstrated the use of a surface antigen targeted approach combined with a particle that can alter the effective density or magnetic susceptibility of cells. Together, the successful enrichment of highly purified, viable cells can be enabled and accelerated in a gentle and effective manner.

References

1. Knowlton et al., Science Reports. 2015;5:15022.

For more information, visit **levitasbio.com,** or contact **sales@levitasbio.com**.