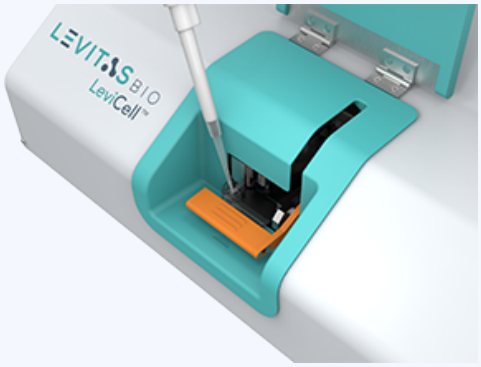
LEVITAS BIO

Stem Cell Enrichment

The Promise of Stem Cell Therapies and How the LeviCell Can Contribute

Introduction

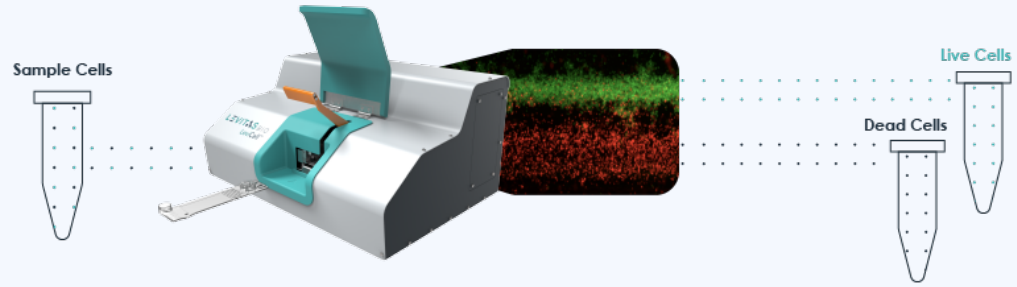
Studying stem cells offers immeasurable promise, potential, and opportunity to advance scientific knowledge. The ability of stem cells to self-renew and differentiate into all functional cell types makes them an ideal tool for understanding human development, drug testing, drug development, tissue engineering, and regenerative therapies. Stem cells are currently used to replace cells damaged by chemotherapy, and researchers are testing ways to incorporate regenerative therapies for cancer, inflammatory diseases, autoimmune disorders, and neurological diseases.



However, successfully culturing stem cells – which are notoriously sensitive - pose challenges that can result in significant additional costs and delays. Integrating the LeviCell platform into stem cell research laboratories as a tool for safely and effectively isolating differentiated cells from culture will enable scientists to generate consistent and reproducible results at a lower cost and shorter timeline than traditional methods.

Methods

To validate the performance of the levitation system, primary samples were analyzed in culture, as well as by qPCR, before and after enrichment with the LeviCell. The LeviCell facilitates a completely touch-free, label-free, three-step cell sorting process that uses less than 1 psi of pressure and takes only 20 minutes to complete. Differentiated stem cells are easily separated from non-differentiated and mis-differentiated cells using the LeviCell. This simplifies the isolation and enrichment process, which saves precious time, minimizes effort, decreases costs, and enhances replicability.



1 Sample Introduction
Sample pipetted into the LeviCell cartridge.

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

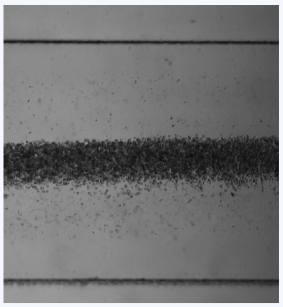
3 Sample Collection
Levitated cells transition into separate collection ports for removal.

Results

Adipocytes

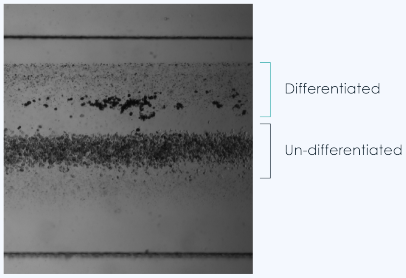
Distinguishing differentiated from undifferentiated adipocyte cells is easily done with the LeviCell due to the density variation between the two cell types. As an example, undifferentiated precursor cells are shown in the first figure below to levitate at a specific position.

a)



Undifferentiated precursor cells levitate at a uniform height, which indicates a homogeneous cell population. These cells were suspended in 100 mM Levitation Agent and allowed to reach their final levitation height shown in Figure a.

b)

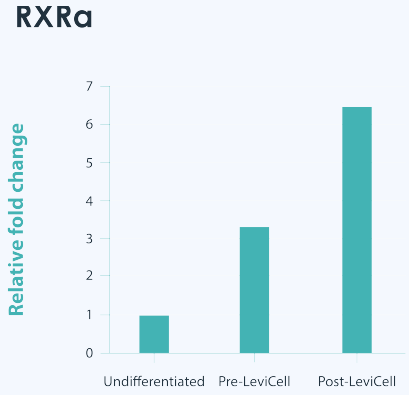


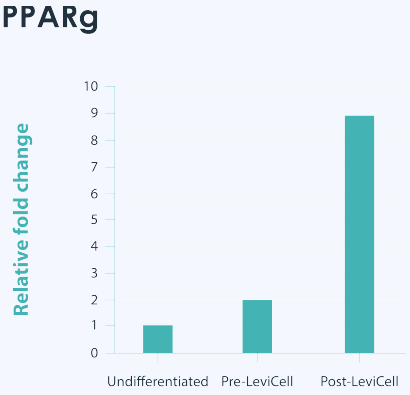
The undifferentiated precursor cells were induced to differentiate into adipocyte cells for 11 days prior to isolation via levitation.

As shown in Figure b, the LeviCell clearly separates differentiated from undifferentiated cells, leaving a clearly discernible line of demarcation between the two cell types. The dark portion of the differentiated/top layer is made up of large cell clusters of differentiated cells that would typically not be available for downstream analysis using conventional cell separation methods. The LeviCell's ability to process cells up to 350 µm ensures minimal loss: of target cells, time, and effort.

Further validation through Gene Expression

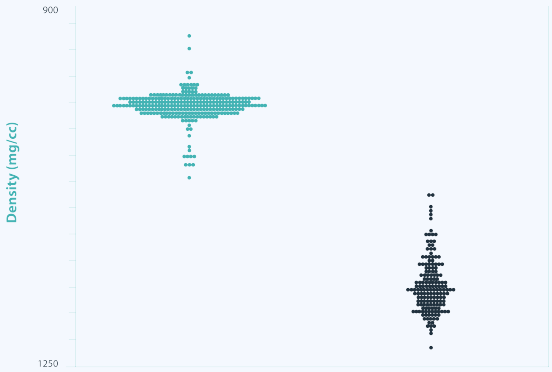
The enrichment was further validated through gene expression analysis where two adipocyte-specific genes RXRa and PPARg were used to identify and quantitate the degree of differentiation in these cells. Undifferentiated, Pre-LeviCell-enriched samples and LeviCell-enriched samples were analyzed by qPCR and the clear enrichment of fully differentiated cells post-LeviCell can be seen in the expression of both of the differentiation-specific genes.





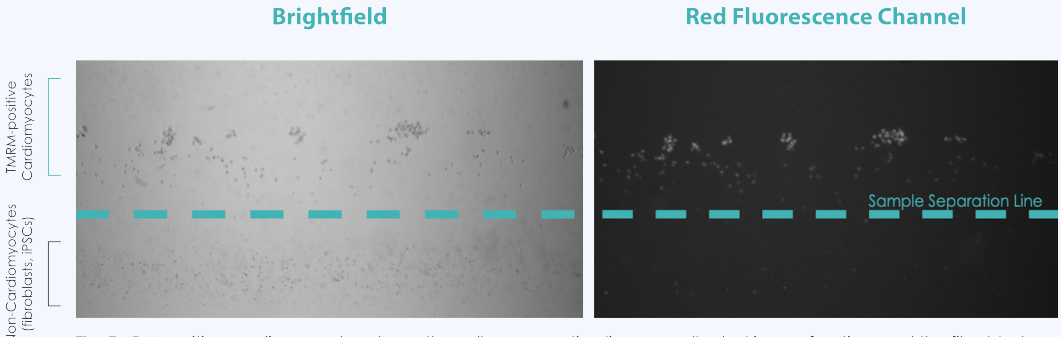
Cardiomyocytes

The LeviCell can easily separate fully viable and differentiated cardiomyocytes from undifferentiated cells and contaminating fibroblasts. In the following example, iPS cells were differentiated into mature cardiomyocytes following a standard protocol as described in "Small Molecule-Mediated Directed Differentiation of Human Embryonic Stem Cells Toward Ventricular Cardiomyocytes" Stem Cell and Translational Medicine, 2014 and analyzed on the LeviCell. The differences in the density of differentiated cardiomyocytes were analyzed and are shown below.



LeviCell significantly enriched for differentiated cells in the top fraction relative to the starting sample, which were differentiated for 11 days.

The cells were then stained with TMRM and enriched on the LeviCell with the top fraction (fully differentiated cardiomyocytes) enriched from the fraction containing non-differentiated cells and contaminating fibroblasts.



The TMRM-positive cardiomyocytes above the yellow separation line are collected in one fraction, and the fibroblasts and iPSC below the separation line are collected in anothe fraction, resulting in high enrichment

Conclusion

Recent advancements in stem cell research and potential treatments based on these breakthroughs have brought this promising branch of science into the scientific spotlight. Ethical concerns about using human embryonic stem cells (hESCs), lower subculture contamination risk, and the significant potential to advance regenerative medicine, drug development, and clinical research, are driving the increased use of induced pluripotent stem cells (iPSCs) in labs throughout the world. A deeper understanding of stem cells is necessary for the development of prospective therapies for many debilitating, chronic, and presently incurable diseases. The LeviCell can help your lab make these discoveries – in less time and with less effort and money.

1455 Adams Drive
Menlo Park, CA 94025
contact@levitasbio.com