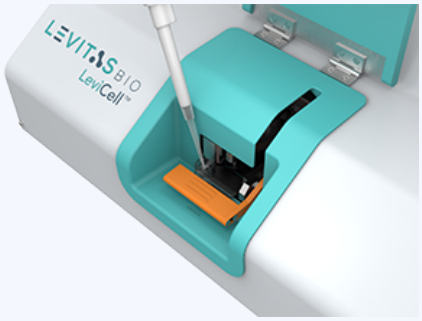
LEVITAS BIO

Adipocytes

The LeviCellTM is a Gentle, Label-Free Levitation Technology Isolates and Enriches Pure Population of Adipocytes in Differentiated Precursor Cell Models

Introduction

The alarming rate at which obesity is growing worldwide has intensified the necessity and immediacy of obtaining a deeper understanding of all aspects of adipocyte biology, as obesity often leads to numerous chronic and costly health conditions. Substantial discoveries have been made in the complex functions of adipocytes that have led to advances in endocrinology, cardiology, energy metabolism, cancer biology, and developmental and stem cell biology. Scientists continue to study the implications of managing adipocyte growth and proliferation in a variety of disease processes to uncover opportunities for unique therapeutic interventions.



The ongoing quest for insight into the varied roles and mechanisms of these cell types puts adipocyte research at the heart of a variety of diseases such as diabetes, hypertension, heart disease, metabolic syndrome, sleep disorders, and cancer. As such, the need to isolate and characterize adipocytes is immense.

However, adipocytes are notoriously problematic to work with. These highly sensitive large cells tend to form bulky clumps that make them challenging to isolate using conventional separation techniques such as FACS. The degree of differentiation in precursor models also poses considerable challenges in assessment and downstream applications due to the limitations in current techniques that leave an abundance of undifferentiated cells in the samples. The LeviCell provides a solution to these limitations.

The LeviCell platform separates cells based on physical properties such as density. Adipocytes have lower density compared to most mammalian cells due to their lipid-rich vesicles. As such, they levitate higher in the LeviCell environment. The nature of the LeviCell technology enables robust enrichment, label-free isolation, and minimal cell loss and damage of these delicate adipocytes.

Since the entire process occurs in a completely closed environment, prep time, contamination potential, and cellular damage as a consequence of repeated handling are minimized. Furthermore, the gentle processing method of the LeviCell, utilizing <1 psi of pressure, accommodates the safe and effective isolation of large cell clumps (up to 400µm) - ensuring maximum viability, even with these highly sensitive cells.

Methods

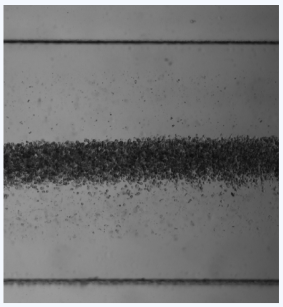
Cell Culture & Differentiation	Cell preparation for LeviCell	qRT-PCR
Used to generate and harvest adipocytes	Used to optimize the selection conditions	Used to quantify differences of genes involved in adipogensys

Results

Cell Differentiation

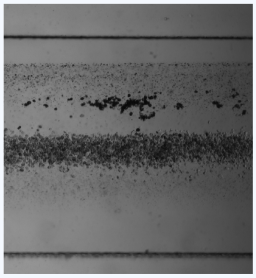
Upon differentiation, adipocyte precursor cells accumulate lipid vesicles that decrease their density. This decrease in density relative to undifferentiated cells is detectable on the LeviCell, and enables cell levitation height to be used as a proxy for differentiation (Fig. 1)

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 1b.

b)

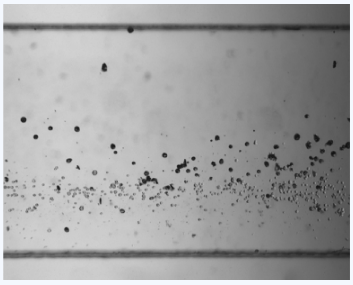


Precursor cells differentiated for 10 days reveal 2 distinct populations. The top layer of cells (blue bracket) indicate differentiated adipocytes, whereas the lower layer (red bracket) are the undifferentiated cells. The dark clumps at the lower portion of the top layer are collections of cells, highlighting the ability of the LeviCell to process large clusters of cells

Cell Preparation for LeviCell

Different concentrations of Levitation Agent were used to determine which would provide maximum levitation distance between the two adipocyte cell populations.

a)



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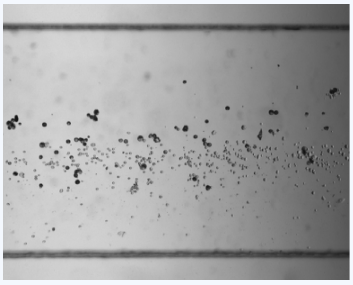

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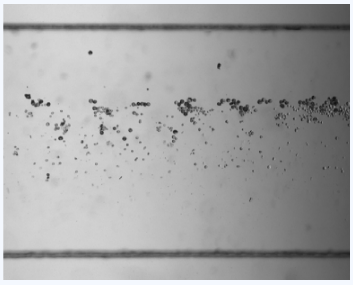
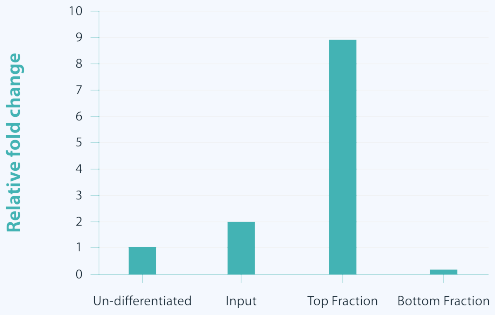


Maximum levitation height difference between differentiated and undifferentiated adipocytes was achieved using 30 mM of Levitation Agent (Figure 2a). Note that as the concentration of Levitation Agent increased, overall levitation height of both populations increased while the distance between differentiated and undifferentiated cells decreased.

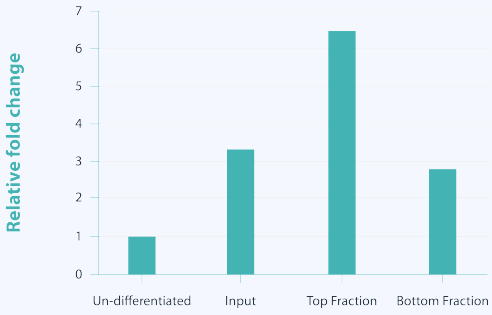
qRT-PCR

After determining the ideal conditions for maximal levitation distance between differentiated and undifferentiated adipocytes, the LeviCell was used to isolate these respective cell populations from a heterogeneous starting sample that had been differentiated for 11 days (Fig 3a,b), and 7 days (Fig 3c,d). qRT-PCR was used to confirm and quantify fold-change enrichment of genes involved in adipogenesis: PPARg (Fig 3a,c) and RXRa (Fig 3b,d) relative to a housekeeping gene (ActinB).

a) PPARg

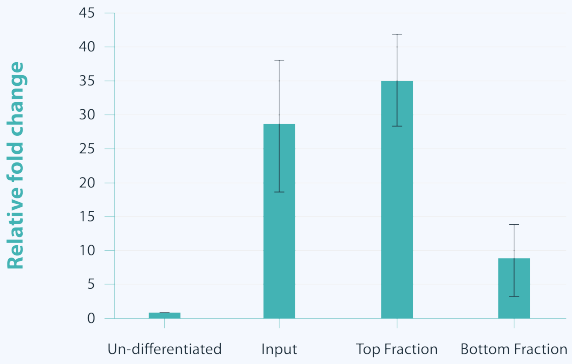


b) RXRa

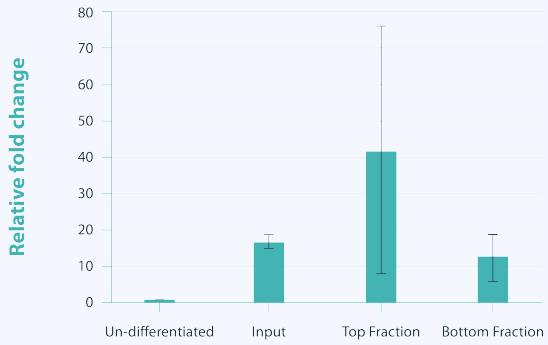


LeviCell significantly enriched for differentiated cells in the top fraction relative to the starting sample, which were differentiated for 11 days.

c)



d)



Starting samples for Fig 3c,d were differentiated for 7 days, indicating that the LeviCell can effectively isolate and enrich sensitive cells such as adipocytes after relatively early differentiation time points.

Conclusion

The LeviCell platform's demonstrated ability to efficiently and effectively isolate large, fragile adipocytes in a gentle, label-free, closed environment shows the platform's immense potential to reset the bar in cell separation technology. Significant advances in understanding adipogenesis, adipocyte-related diseases, and fat tissue engineering are within reach as the LeviCell unambiguously exhibits its ability to enrich an abundance of pure and viable adipocytes in a shorter time span than conventional techniques.

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