Enriching for viable organoid human cells with Levitation technology

Overview

Using organoid lines derived from human adult stem cells has been established as a good model that recapitulates important aspects of in vivo organs. The fact that they can be derived from healthy or diseased epithelium of multiple organs expands the field of personalized medicine and opens doors to future treatments[1]. Moreover, organoids can be paired up with any experimental approach that has been developed for cell lines making this model a powerful tool for laboratory and clinical research.

Maintaining a precious organoid line is critical as it may have been derived from the only available sample from an actual patient. Loss of cell viability through multiple passages can endanger and setback the work for months. Robust organoid lines rely on viable cells present in the culture with each passage and their ability to replicate and expand the culture. Maximizing viability and cell health is integral when reestablishing older lines that have been cryopreserved. Often organoids, similar to traditional cell lines, are highly sensitive to the freezing and thawing cycles which can directly impede their ability to grow successfully.

Elevate Organoid Work with the LeviCell System

The LeviCell system, a label-free and bias-free solution for sample processing, addresses all of these challenges through fast, simple, and extremely gentle viable cell organoid enrichment. Once the organoids are dissociated into single cells, they can be enriched for viable cells using levitation. This simple 3-step workflow can supplement organoid workflows in the following ways:

- Selection of viable cells from your cryopreserved organoid vials before culturing
- Maximizing percentage of viable cells and minimizing dead cells during passaging
- Isolation of your drug resistant organoid cells
- Collection of transfected viable cells to grow your transformed organoids
- Separation of infected organoid cells from uninfected cells

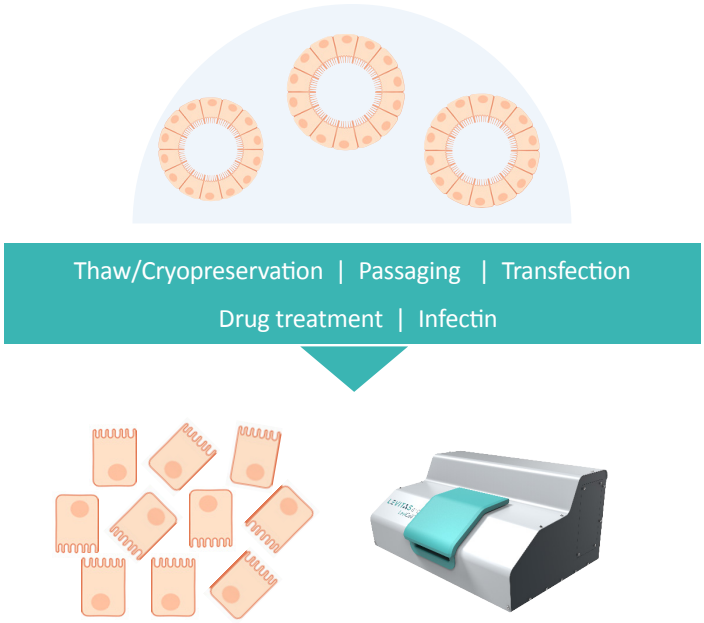

Figure 1. Different use cases for organoids using LeviCell technology. Organoids grown in BME (Basement Matrix Extract) can be dissociated into single cells for different uses. The LeviCell technology enables enrichment for cells of interest for downstream workflows. For more information about the protocol used to generate these organoids see Sato et al[2]. Part of this figure was created with BioRender.com.

Maintenance of 3D Structure through Gentle Levitation

Organoids demonstrate a high order of self-assembly that allows a better representation of complex microtissue as compared to 2D cell cultures. The scaffold in which the organoids grow allows them to assemble into 3D models and establish crucial cell-cell and cell-matrix interactions. LeviCell capabilities offer the possibility to work with whole organoids up to 350µm in diameter. Human gastric organoids derived from adult stem cells were introduced into the LeviCell to separate in 5 min the ones alive from the ones composed mostly of dead cells (figure 2). Apart from this separation, the low pressure applied did not affect the 3D structure of the organoids when collected for further use (figure 3).

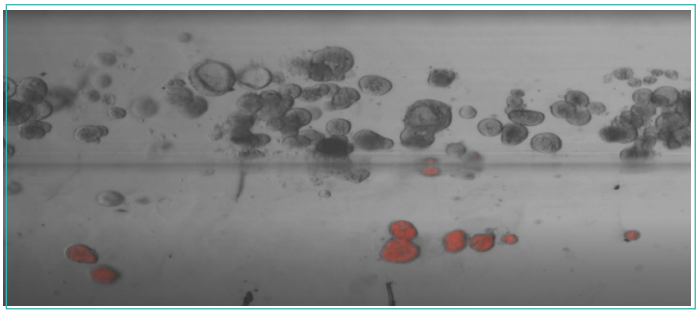

Figure 2. Organoids levitating inside the LeviCell. Viable organoids can be seen here in the upper part of the channel, while dead organoids (stained with PI for visualization purposes only) can be seen in the lower part of the chamber.

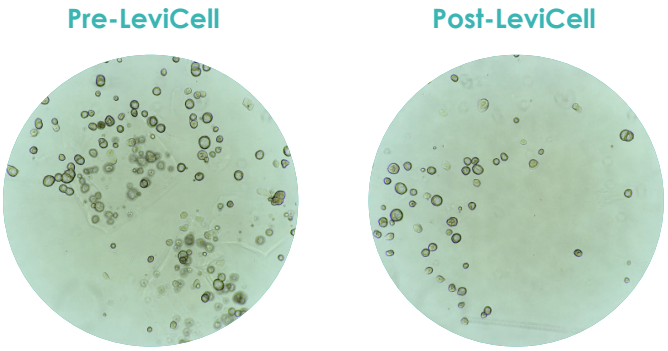

Figure 3. Levitation does not affect the spherical form of the whole organoid. Left image shows gastric organoids inside their Basement Extract Matrix after 7 days of growth, and the right image shows the organoids after 20 minutes of levitation on LeviCell.

Maximize cell viability from frozen vials or routine passaging

Organoids, like cell lines, can be thawed from frozen vials and need to be passed routinely to maintain a stable population. These processes rely on the viability of the organoid cells and their potential of replicating and forming new organoids again. LeviCell technology has demonstrated multiple times the enrichment of live cells from a given sample. In this case, gastric organoids grown in their scaffold for 7 days were dissociated into single cells and run on the LeviCell. After live cell enrichment, most of the live cells were collected on the top fraction, providing viabilities in some cases greater than 90% (figure 4). That highly viable fraction is ready to initiate a new culture as it is free of dead cells and debris.

Thank you:
The laboratory of Professor Manuel Amieva at Stanford University graciously provided samples for this study.

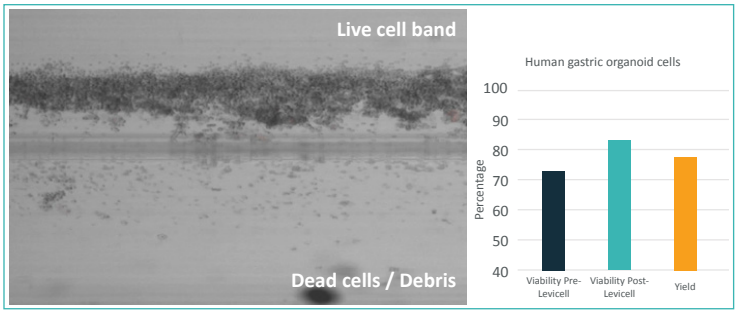

Figure 4. Enrichment of viable organoids on the LeviCell. Two distinct bands of cells are observed, with viable organoids levitating higher and dead cells and debris levitating lower (left). After 20 minutes of levitation, the viability of the organoids improved from >70% to >80% with a final yield of just under 80%. The cell input ranged from 25K-160K cells, n=3. (right)

Separation of Specific Cells Using Levitation

As LeviCell technology provides a fast and easy separation of live and dead cells, it can be used to sort the resistant cells of an organoid culture after a drug treatment for further analysis and characterization. This can have a great impact in cancer research where patient-derived organoids are being tested for different drugs to get some insight of the drug effect and guide their treatment. At the same time its use after organoid cells have been transfected can increase the proportion of viable transformed cells before culture.

While viable cell enrichment is a powerful application in organoid research, LeviCell technology can be expanded into more specific fields. Some pathogens affect the metabolic state and cell composition of the infected cell provoking a change in its intrinsic density. That difference can be ob-served on the LeviCell and used to separate the infected from the uninfected cells.This can be extremely useful for the study of specific infection activated pathways and to uncover targets for possible treatments.

In a novel and highly gentle way, the LeviCell System and Levitation technology can be the choice method for extending organoid research and accelerating discovery insights of disease.

References:
1.Kim, J., Koo, BK. & Knoblich, J.A. Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 21, 571–584 (2020). https://doi.org/10.1038/s41580-020-0259-3
2.Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011 Nov;141(5):1762-72. doi: 10.1053/jgastro.2011.07.050. Epub 2011 Sep 2. PMID: 21889923.

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