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3D Cell Culture Tips & Tricks: Zonal analysis of spheroid physiology with SPAchip® in real-time

In the realm of drug discovery, three-dimensional (3D) cellular spheroids have emerged as a robust model for high-content screening (HCS) approaches. Unlike traditional two-dimensional (2D) cultures, spheroids better emulate the in vivo environment, offering more reliable and physiologically relevant data. This blog delves into the applications of 3D cellular spheroids, detailing their production, and highlighting various assays and readouts critical for drug screening.

The Relevance of 3D Cellular Models

3D cellular spheroids offer a more realistic model for studying cellular behavior due to enhanced intercellular communication and interaction with the extracellular matrix (ECM). In 3D cultures, cells maintain their natural morphology, polarity, and cell-cell junctions, which are often disrupted in 2D cultures. The ECM in spheroids provides structural support and biochemical signals, influencing cell differentiation, proliferation, and survival.

Regional Differences in Cellular Activity

Spheroids exhibit distinct regions with varying cellular activities due to their microenvironment:

- **Proliferating Shell:** The outermost layer of the spheroid, where cells are exposed to abundant nutrients and oxygen, supports active proliferation.
- **Quiescent Viable Zone:** Located beneath the proliferating shell, this region contains cells that are viable but not actively dividing. Reduced nutrient and oxygen levels induce a quiescent state.
- **Necrotic Core:** At the center of larger spheroids, limited oxygen and nutrient diffusion lead to cell death, forming a necrotic core.

These regions are determined by gradients of nutrients, oxygen, and metabolites. Cells in the proliferating shell have high access to these resources, while cells in the core experience hypoxic and nutrient-deprived conditions. This zonal architecture mimics the tumor microenvironment, providing insights into drug penetration and efficacy.

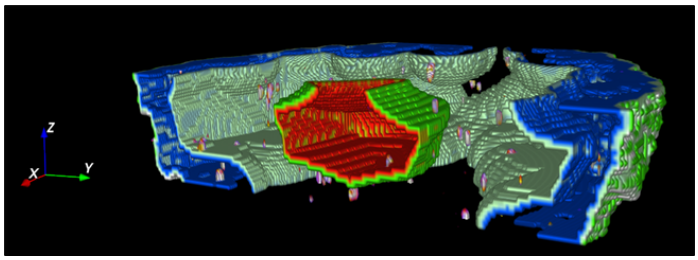

Figure 1. 3D Volumetric representation of a spheroid lower section obtained by confocal microscopy. Regional analysis has been depicted to quantify SPAchip penetration during time-lapse experiments.

Key Fluorescence Microscopy Readouts for Spheroid Analysis

To fully leverage the potential of spheroids in drug screening, multiple assays and SPAchip readouts can be employed to monitor different aspects of cell behavior.

- **Cell Aggregation Monitoring:** Time-lapse fluorescence microscopy is used to observe the formation of spheroids over time. Spheroid Size and Geometry helps in assessing the uniformity and structural integrity of spheroids,
- **Cell Viability and Death:** fluorescent dyes indicating metabolic activity and cell viability are essential for determining the overall health and viability of spheroids under different drug treatments. Early apoptotic cells or necrotic cells can be also identified.
- **Functional Readouts:** fluorescence staining enables the detection of different intracellular molecules providing information about specific signaling pathways involved in drug effectiveness.
- **Mitochondrial Health:** pH measurements assess the mitochondrial environment, while Reactive oxygen species (ROS) and intracellular oxygen levels can be evaluated to study mitochondrial respiration, providing comprehensive insights into mitochondrial function, oxidative stress and cellular metabolism.

Conclusion

The integration of advanced fluorescence microscopy and quantitative analysis of SPAchip(R) readouts provide a more accurate representation of in vivo conditions, enhancing the predictive power of drug screening assays. Regional analysis can exponentially increase the predictive power of our 3D spheroid models.

It all translates into saving costs and time, and increasing relevance, quality and versatility

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