DECIPHERING THE INSIDE CELL´S STORY BY CYTOCHECK SPACHIP®!

SPAchip® technology enables the study of calcium signaling in receptor pharmacology.

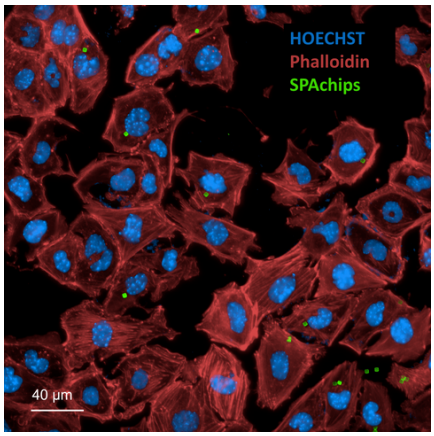

Figure 1. HL1 cells micrograph at 40X with calcium green detection SPAchip® internalized.

Calcium ions (Ca2+) are one of the most important signal transducers in cells, hence intracellular levels must be tightly regulated since prolonged high levels can lead to cell death. Since extracellular calcium concentration is usually 20,000-fold higher than intracellular levels (i.e. 1-2 mM cf. 100 nM), cells have developed several mechanisms to keep its homeostasis. Calcium ions can get into the cytosol from the extracellular space through ion channels or be pumped from the endoplasmic reticulum (ER). This organelle stores these ions in its lumen by specialized buffer molecules which will release calcium whenever needed and correctly signaled.

Excitable cells possess selective **calcium channels** which suffer conformational changes by depolarization of resting membrane potential. Then, calcium can enter the cell triggering actions, such as exocytosis in neurons or contraction in muscle, by inner surface membrane proteins. **Non-excitable cells** such as hepatocytes or endothelial cells use **G protein-coupled** receptors which triggers intracellular increase of calcium levels from the ER through InsP3, i.e. inositol (1,4,5) trisphosphate, as a second messenger.

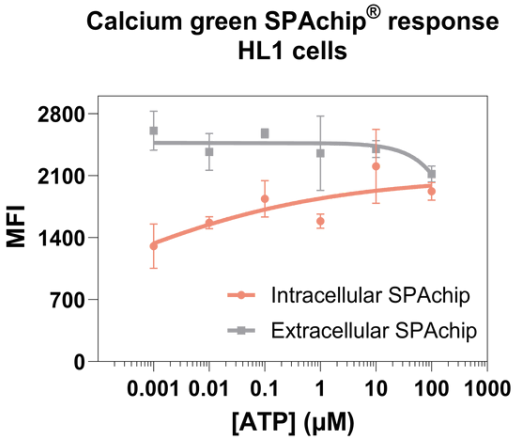

Figure 2. SPAchip® response to calcium concentration with the addition of ATP, agonist of purinergic receptors, in the culture media.

The InsP3 receptor can be modulated by Mg2+, ATP and Ca2+. **ATP is an agonist of purinergic receptors promoting the release of calcium ions via InsP3**.

This is depicted in Figure 2, where the addition of ATP in the cell culture media triggers the release of intracellular calcium levels in comparison to extracellular space which remains constant. Thus, the analysis of the implications of intracellular calcium in cell signaling can provide with valuable information in receptor pharmacology.

SPAchip® advantages in contrast with current methods:

- **Compatibility** with other assays such as live cell painting or other markers. SPAchip® only remains as a small fluorescence spot inside the cytosol enabling its usage with other staining. Likewise, it is compatible with different **fluorescence HCS readouts**, confocal microscopy, or flow cytometry.
- **Reliability outputs** since the small spot are in the cytosol which avoids disturbance from another organelle staining when treating the whole cell.
- **Long-term assays**: SPAchip® technology is **non-toxic** for several weeks, even mixing different readouts after calcium measurement.
- It allows **single cell measuring and tracking** since it is harmless and do not alter cell function and expression.
- Available for **wide variety of cell cultures** from 2D monolayers to 3D cell cultures or suspension cells.
- **Real time kinetic analysis**. Its measurement does not comprises only end time points of the experiment. Also, several measurements can be performed following the sequential addition of agonists and antagonists in the same experiment.

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

Bibliography:
- Lysosomal cell death is controlled by pH-dependent calcium channels (https://doi.org/10.1126/sciadv.abe5469).
- Control of calcium homeostasis in mitochondria is dependent upon extracellular pH (https://doi.org/10.1074/jbc.M411507200).
- Mitochondrial proton gradient is regulated by cytosolic calcium signals (https://doi.org/10.1007/s00424-012-1106-y).
- Extracellular acidic environment induces apoptosis through an augmentation in intracellular calcium level and generation of endoplasmic reticulum stress, without any contribution of reactive oxygen species (ROS) (https://doi.org/10.1007/s12192-014-0568-6).

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