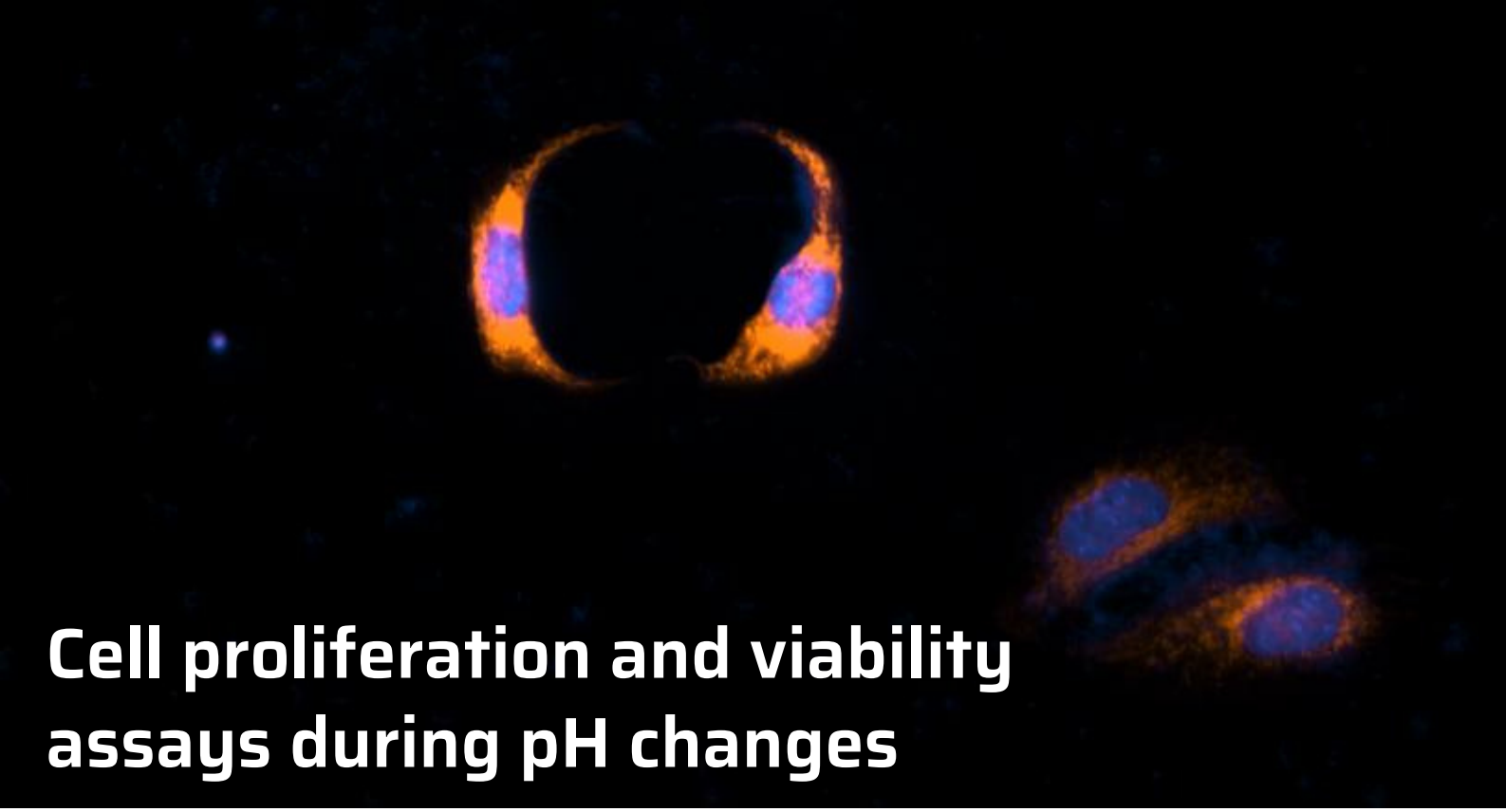

Cell proliferation and viability assays during pH changes

Cell proliferation is an increase in cell number due to cell division, or cytokinesis. Cell viability measures the percentage of healthy cells within a population. Both processes are essential for normal tissue development and maintenance over the lifespan.

Cell proliferation and viability assays allow researchers to study the metabolic activity of cells after drug treatment or after exposure to stimuli or toxic reagent. These experiments detect changes in the number of cells in division, changes in cell population and/or evaluate cell conditions. Additionally, detecting changes in cell growth can give us an idea if the cell culture has been contaminated or undergone genetic drift.

In conclusion, cell proliferation and viability assays are essential tools in cellular biology and drug discovery. Optimization of both cell proliferation and cell viability assays is crucial taking into account that number and type of cells can vary among experiments. Overall, these cell-based assays allow to measure cell survival over time in different cell conditions, specially important during drug screening.

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01 CELL PROLIFERATION ASSAYS

Cell proliferation is a highly regulated process where specific proteins directly or indirectly control cell cycle checkpoints. Several diseases, such as cancer, present genetic mutations, causing uncontrolled cellular proliferation.

02 CELL VIABILITY AND CYTOTOXICITY

Cell viability assays allow evaluation of metabolic activity and cell status in response to different compound treatments or toxic agents. SPAchip® cell based assays in combination with other viability cell assay techniques can be carried out to better explore the condition of cell population as well as changes in intracellular pH.

03 METABOLIC ACTIVITY

Together, cell viability and cell toxicity assays are important tools for assessing metabolic activity of a cell population. This allows to better evaluate cellular responses to specific experimental compound treatments.

04 RESEARCH APPLICATIONS

Drug sensitivity, cytotoxicity, cell activation, optimization of cell culture, and compound testing. Cell proliferation and viability assays are reproducible, extremely sensitive, and compatible with high-throughput screening (HTS) techniques.

Cell proliferation and viability assays with CytoCHECK SPAchip® pH Single-Detection Kit

Advanced image and quantitative analysis to investigate the metabolic activity, cell condition, and pH changes during drug testing

CytoCHECK SPAchip® pH Single-Detection Kit allows measurement of intracellular pH levels by changes in fluorescence intensity, which facilitates a more comprehensive study of the living single-cell physiology and maximizes the performance of most of imaging analyzers.

In addition, cell proliferation and viability can be monitorized by using live/dead cell staining. Incubating with both our SPAchip® technology and another commercial dye, estimated cell number per well decreased in cells incubated with commercial dye at 24, 48, and 72 hours after treatment (Fig. 1), whereas cells incubated with SPAchip® displayed similar cell number to control conditions (Fig. 1), proving that SPAchip® does not offer cytotoxic effects in terms of proliferative capabilities.

Cell proliferation assays allow to measure cell division and live/dead cell count as well as to facilitate the evaluation of cytotoxicity. Cell viability assays give insights into the overall health of cell culture population. Both cell-based experiments enable measurement of cell survival following compound treatment, gaining useful information for drug testing.

Using live/dead cell staining together with our cutting-edge SPAchip® technology opens the door to combining functional readouts with cell conditiona diverse set of cellular assays. Thus, the ability to visualize cell proliferation and cell viability together with tracking pH changes using both technologies opens up new avenues for developing targeted therapies for several diseases. In conclusion, the combination of these powerful technologies is a game-changer in the field of cell-based experiments since it allows to measure cell survival, viability and pH changes following treatments with compounds, such as during drug screening.

Experiment setup:

- **Cell lines:**
  - **HeLa** (human cervical carcinoma cell line)
  - **HEK293** (kidney; embryo)
  - **SH-SY5Y** (neuroblastoma cells)
  - **CAL-51** (breast carcinoma)
  - **MDA-MB-231** (epithelial-like cells; breast mammary gland)
  - **ARPE-19** (retinal pigment epithelia)
  - **HL-1** (cardiac muscle cell line)
  - **1095SK** (fibroblast)
  - Any other cell type of your choice
- **Fluorescent dyes:**
  - Nuclei staining
  - Live/Dead cell staining
  - CytoCHECK SPAchip® pH Single-Detection Kit Green or Red
- **Positive and negative controls**
- **Imaging over time at different time points**
- **Measurements:**
  - Total cell number
  - Live cells
  - Dead cells
  - % live vs dead cells
  - Cell viability

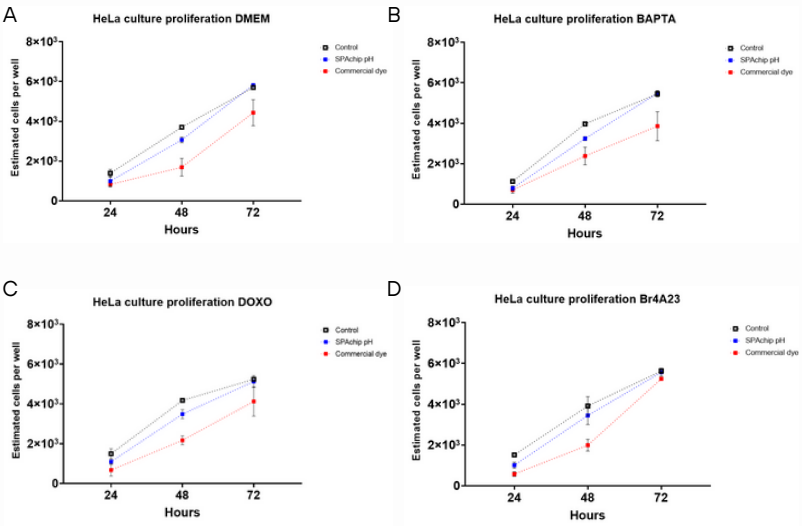

Figure 1: Estimated cells per well in HeLa cells incubated 24, 48 and 72 hours in basal conditions **A)** BAPTA **B)** and DOXO **C)** and Br4A23 **D)**. For each time measurement, three conditions were evaluated 1) control cells with no treatment, 2) cells incubated with CytoCHECK SPAchip® pH Green Single-Detection kit and 3) cells incubated with commercial dye. Bars represent mean values for each condition and error bars correspond to SD.
Estimated cells per well decreased in cells incubated with commercial dye at 24, 48 and 72 hours, showing that commercial dye affected cell proliferation and viability. Cells incubated with CytoCHECK SPAchip® pH Green Single-Detection showed similar number of cells compared with control.

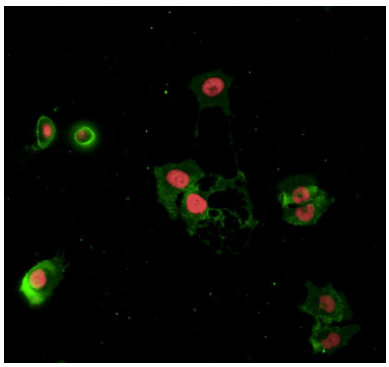

Figure 2: HL-1 cardiac muscle cell line stained with DRAQ5 in red and CellMask in green. Number of live and cells can be measured and analyzed for cell proliferation and viability studies.

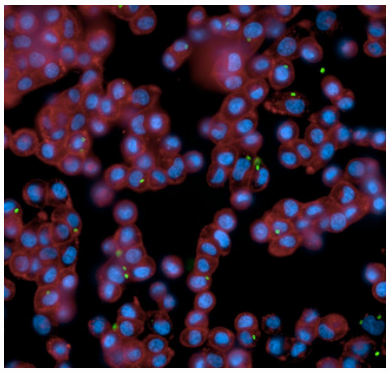

Figure 3: HeLa human cervical carcinoma cell line stained with Hoechst in blue and CellMask in deep red. CytoCHECK SPAchip® pH in green allow measurements of pH changes.

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