Calcium Measurement with CytoCHECK SPAchip® Calcium Single-Detection kits

Intracellular Calcium Response Analysis in Living Cells of Different Type by new Lab-in-a-Cell Devices based on Spachip® Technology.

BACKGROUND

Calcium ions (Ca2+) play a vital role in the functioning of living cells. They are involved in a wide range of physiological processes, including muscle contraction, neurotransmitter release, and insulin secretion [1]. There is a need to be able to analyse the intracellular Ca2+ signaling processes from milliseconds to hours to days. For this reason, living single cell analysis needs dynamic, real-time assays that resemble the native conditions of the cell while maintaining its physiological conditions over time [2].

CytoCHECK SPAchip® assay kits are novel fluorescence assays developed by A4Cell that bring together the fields of nanotechnology and cell biology. CytoCHECK SPAchip® kits are composed of silicon microparticles -SPAchip®[3]- that can be internalized in the cytosol of cultured cells and monitor changes for long periods of time. CytoCHECK SPAchip® kits are optimized for its use with fluorescence microscopes and HCS/HCA platforms with confocal capability, yet epi-fluorescence microscopes and imaging system with fixed wavelength filters can be also used.

Cellomics reagents can be used in drug discovery campaigns to analyze intracellular Calcium levels. Relevant information on the role of calcium signaling can help to elucidate the Mechanism of Action of different molecules. CytoCHECK SPAchip® Calcium Single-Detection kit allows to perform real-time dynamic cell-based assays by fluorescence intensity measurements, which allows a more comprehensive study of the cell physiology processes where Calcium signaling is involved and maximizes the performance of imaging analysers.

GOAL

To demonstrate dynamic Calcium monitorization in living single cells under different treatment conditions (dose-response curves) by using SPAchip® nanodevices following the CytoCHECK SPAchip® procedure.

Array for Cell Nanodevices SL
CAIT-UPM. Parque Científico y Tecnológico. Campus Montegancedo s/n
28223-Pozuelo de Alarcón (Madrid)- info@a4cell.com

MATERIALS

- CytoCHECK SPAchip® Calcium Single-Detection Kit
- Tissue-culture treated 384-well imaging plates (PerkinElmer)
- Mia PaCa-2 cells (CRL-1420, ATCC), SHSY5Y cells (CRL-2266, ATCC)
- DMEM cell culture medium(Gibco) supplemented with FBS, gentamycin, L-Glutamine.
- Operetta CLS (PerkinElmer)

METHODS

1. **CytoCHECK SPAchip® Calcium-Detection Kit preparation:** Assay SPAchips were dissolved in Assay Buffer contained in the kit, centrifuged, pelleted and resuspended again in Assay Buffer following A4cell's procedure.
2. **Cell Culture:** Mia PaCa-2 and SHSY5Y cells were seeded independently in duplicates in a 384-well plate by adding 2500 cells in 30 µl medium and allowed to adhere for 24h.
3. **SPAchip® addition:** a SPAchip®-to-cell ratio of 2:1 was added to each well by diluting 5000 SPAchips in 20 µl of fresh medium. Cells were incubated overnight to allow them to uptake the SPAchips.
4. **Drug Treatment:** Dose-response curves with Doxorubicin were prepared by serial dilution (1:10) ranging from 0.05 nM to 5 µM.
5. **Imaging:** Multiwell plate was imaged in an Operetta CLS with a 40x water immersion objective of 6 fields per well. SPAchip® fluorescence was imaged a standard GFP channel (Ex: 488 nm, Em: 520 nm). Digital phase contrast was used to identify cells. All samples were analyzed at 24h, 48h and 5 days after treatment.

RESULTS

Fluorescence microscopy images of pancreatic cancer Mia PaCa-2 and neuroblastoma SYHY5Y cells were analyzed and segmented using GFP and digital phase contrast channels to locate intracellular SPAchips and measure their relative fluorescence intensity units (RFU). Sample data were normalized to control conditions of untreated cells at 24h, 48h and 5 days after drug treatment (**Fig.1 A**).

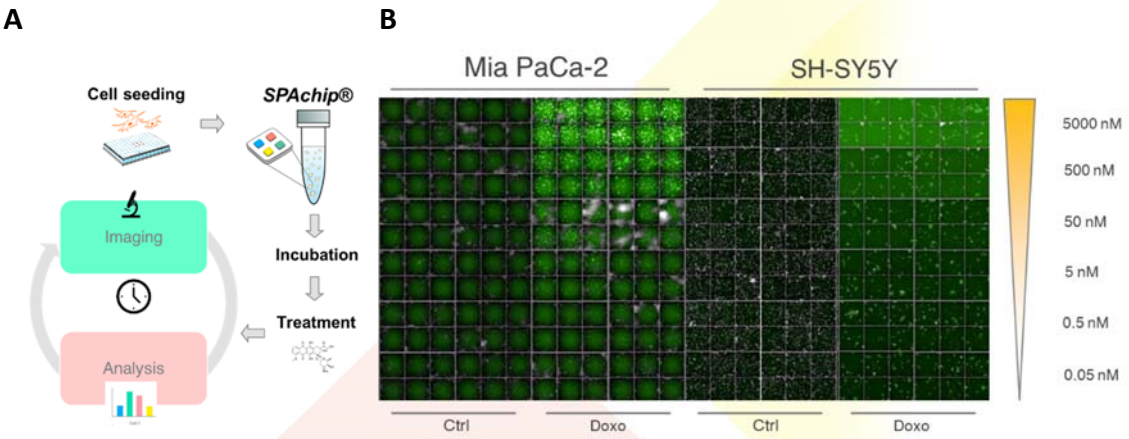

Fig 1: CytoCHECK SPAchip® Calcium Single-Detection use in Drug Discovery Applications. A) Diagram showing the cell assay procedure to perform dynamic analyses in real time by fluorescence microscopy. **B)** Multiwell Plate design showing 6-field montages of each well for Mia PaCa-2 and SH-SY5Y cells at 40x magnification, including merged images of green-fluorescent channel and digital phase contrast. Doxorubicin treatments where applied in 10-fold dilutions from 0.05 nM to 5 µM.

Calcium signal response ranges from 0.05 nM to 5 µM in physiological conditions. Here, we observed how higher concentrations of Doxorubicin indicate an increased calcium concentration upon treatment in both cell lines. There was a cumulative effect of the treatment in Mia PaCa-2 and SYHY5Y cells calcium signals, reaching a 50% and 80% increase respectively at 5 days post-treatment (**Fig. 2**).

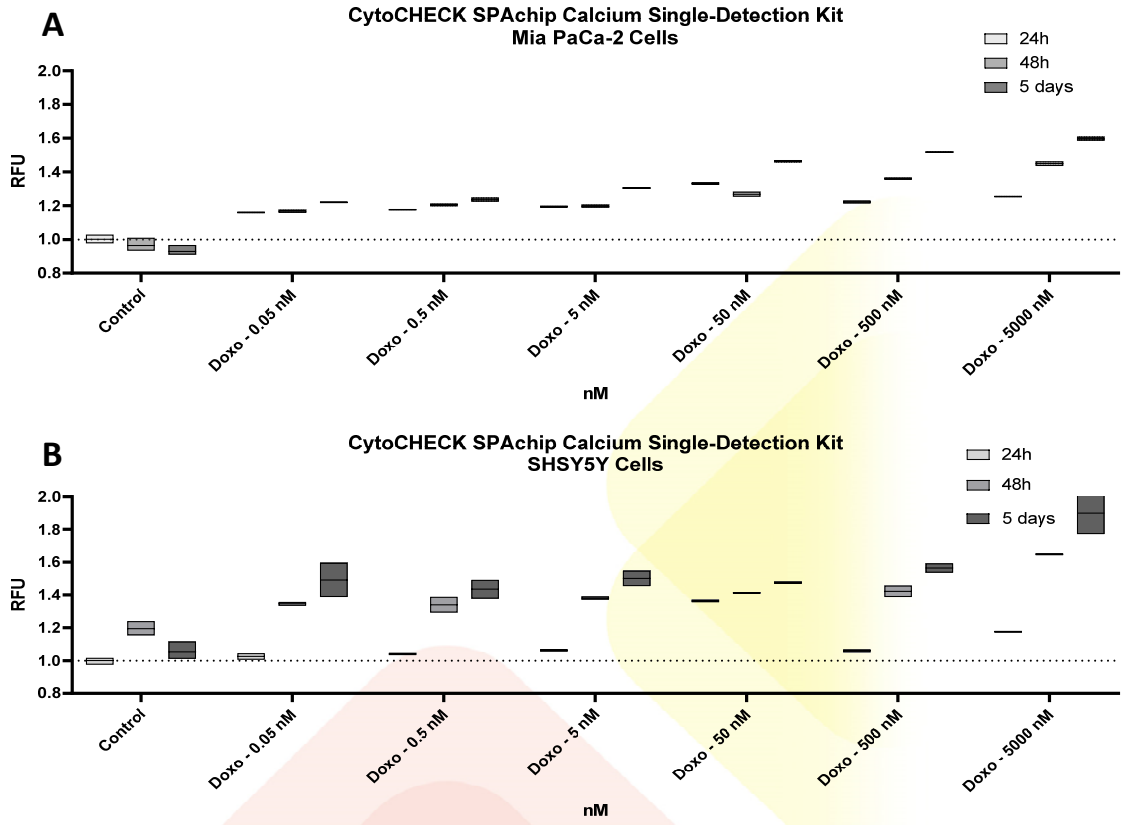

Fig. 2. CytoCHECK SPAchip® Calcium Single-Detection kit analysis by fluorescence microscopy. Effect of Doxorubicin treatment on normalized intracellular Calcium levels of increasing concentrations at 24h, 48h and 5 days after treatment on **A)** Mia PaCa-2 cells and **B)** SHSY5Y cells measured by fluorescence microscopy.

CONCLUSIONS

Novel tools for living cell analysis are needed to offer the possibility continuous monitoring. CytoCHECK SPAchip® kits have been designed to remain in the cytosol without producing cytotoxic effects, thus, allowing dynamic cell analysis in real time. In combination with fluorescence microscopy imaging, we can obtain basic cellular information including morphological parameters, as well as intracellular calcium readouts. CytoCHECK SPAchip ® Calcium Single-Detection kit has been validated as a powerful tool to be used as a lab-in-a-cell device. This information can be used to shed light on the mechanism of action of compounds during drug discovery campaigns.

REFERENCES

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SPAchip® technology is the best available tool to detect Calcium by fluorescence microscopy analysis in HCS platforms after several days in culture at different time points during early drug discovery processes

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