Detecting intra- and extracellular pH changes after mitochondrial inhibition by flow cytometry with “CytoCHECK SPAchip® **pH detection kit**”

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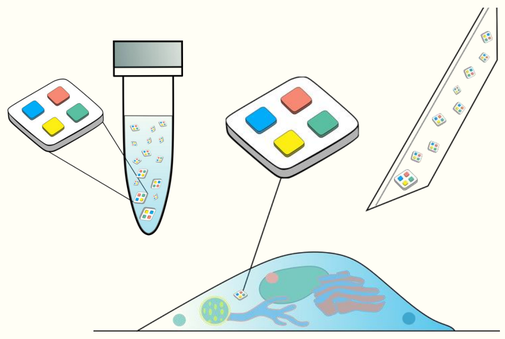
OVERVIEW

- A4Cell presents SPAchip® technology for living single-cell analysis
- SPAchip® technology features absence of cytotoxicity
- CytoCHECK SPAchip® pH detection kit allows measurement of intracellular and extracellular pH in cell cultures
- CytoCHECK SPAchip® Detection kits are compatible with fluorescence microscopy and flow cytometers

INTRODUCTION

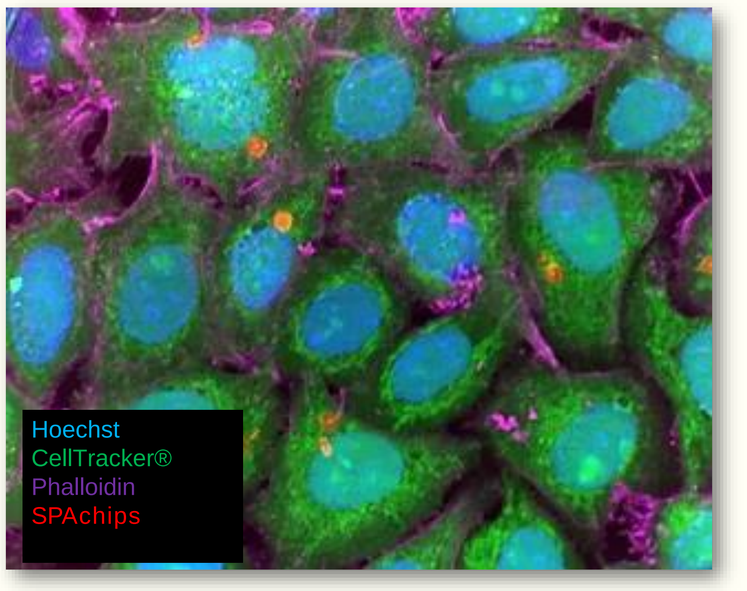
Many cell processes, as those regulating metabolism or proliferation, are highly sensitive to pH changes. For instance, enzymatic activities are generally optimal over a narrow pH margin and heavily decrease above and below it. In this regard, net charge and structure of macromolecules also depend on proton concentration and maintaining intracellular proton fluxes is crucial for energetic metabolism. On the other hand, alterations in cell metabolism also induce changes in cytosolic and extracellular pH. Thus, Warburg effect in cancer cells leads to increased glucose uptake and lactic acid production, which in turn alters extracellular and intracellular pH. Therefore, developing new tools for measuring proton fluxes in cells is appealing for a better understanding of cell physiology.

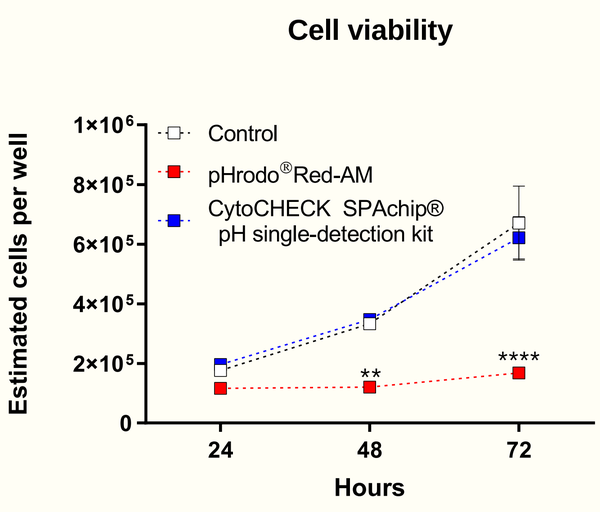


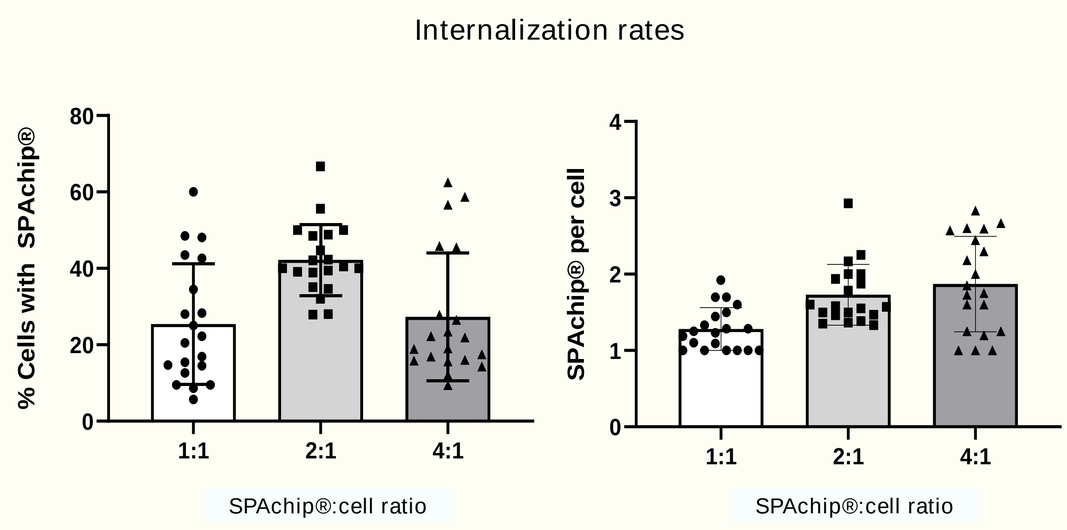
SPAchip®: An intracellular silicon device on which multiple highly concentrated fluorescent probes can be printed to provide intracellular readouts over long culture periods

OUR TECHNOLOGY

Here we feature the CytoCHECK SPAchip® pH single-detection kit for simultaneous measurement of cytosolic and extracellular pH in fluorescence microscopy and flow cytometry (FC). SPAchip® are small (3x3x1 µm) silicon oxide chips functionalized with a fluorescent pH-sensing probe. By diluting SPAchips directly in cell cultures, they get internalized by cells after an overnight incubation and pH changes can be tracked in living single cells by measuring fluorescence intensity during extended periods of time. When added to cell cultures in a ratio 2:1 (chip:cell), an average >25% of the cells internalize a chip in the cytosol, where they remain for days without altering cell viability or losing performance.







PROOF OF CONCEPT

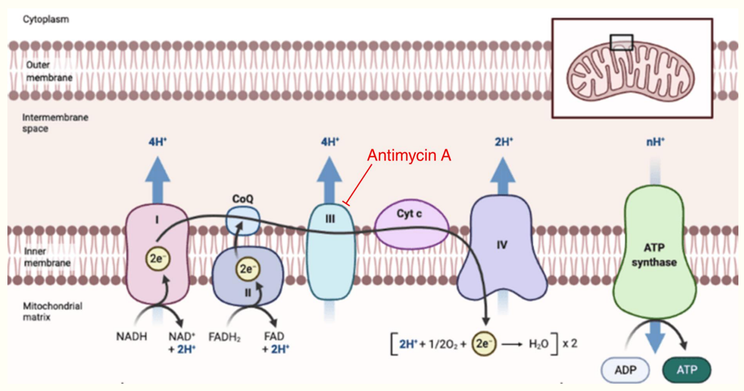
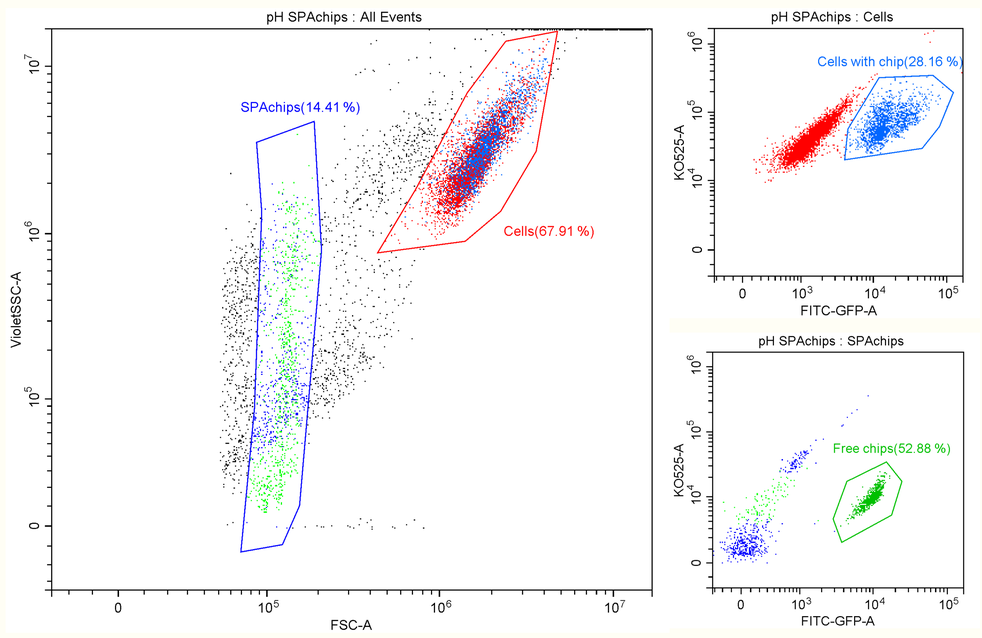
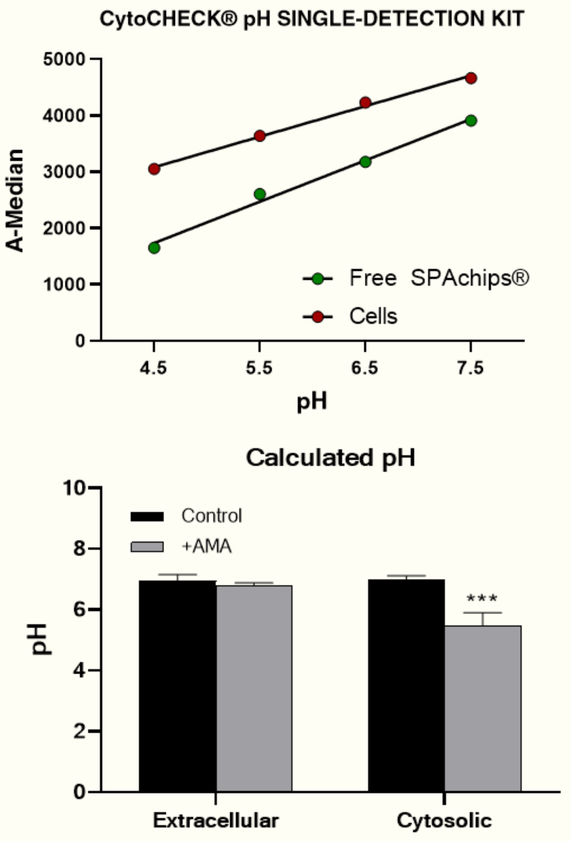


Image modified from: https://researchtweet.com/mitochondrial-electron-transport-chain/

CytoCHECK SPAchip® pH-single detection kit was used in 293T cells incubated in presence or absence of Antimycin A (AMA) for 15 minutes. After calibrating the system with cytosolic pH calibrators, a significant decrease in cytosolic pH was observed in cells treated with AMA, with minor changes in cell medium. Bars represent the mean ± SD of two experiments in triplicate. Statistical comparison vs Control ***p<0.001 (Sidak test).





CytoCHECK SPAchip® pH-single detection technology is a new reliable and accurate tool for performing cell analysis in flow cytometry. Its capability to detect pH changes in the cytosol and cell environment simultaneously makes our technology a valuable tool for:

- Basic cell biology and cell physiology studies.
- Quality Control in bio-industrial processes based in cell cultures, where controlling extracellular pH affects product yield, as recombinant antibody or AAV production.