1000-A: Design and validation of SPAchip® microdevices for continuous intracellular ROS monitoring in living single cells

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Overview

- ✓ ROS SPAchip® enables the measurement of intracellular reactive oxygen species without inducing any cytotoxic effects on the cells.
- ✓ The selective detection of OH· by ROS SPAchips has been demonstrated by chemical assays.
- ✓ Variations in pH have been investigated in relation to the fluorescence signal in order to mitigate false positives and assess the specificity of the SPAchips.
- ✓ Bio-validation performed on cells has revealed the fluorescence of SPAchips upon internalization after a 6-hour incubation period.

Introduction

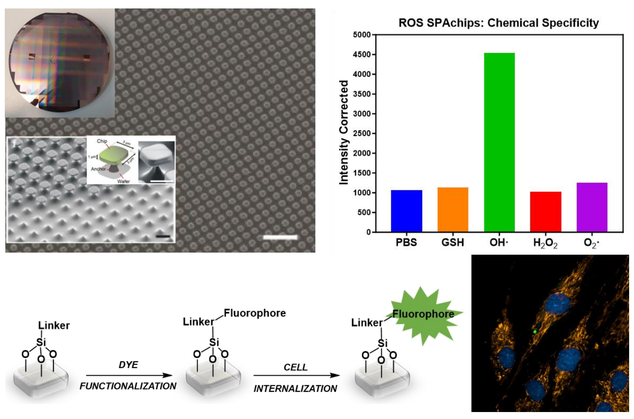
- ✓ Reactive oxygen species (ROS) are commonly used to define the reactive molecules and free radicals originating from molecular oxygen. A large number of studies have proved the messenger role of ROS in cell survival, proliferation, differentiation, death and apoptosis.
- ✓ Different levels of ROS have been demonstrated to play distinct roles in cell life: low concentrations could stimulate the mitosis and proliferation of cells, while a moderate dose would inhibit cell cycle and, with increase of their concentration, apoptosis might be activated.
- ✓ SPAchip® technology facilitates surface modification with a fluorescent dye, enabling real-time monitoring of intracellular ROS through population to single-cell analysis. Here, we demonstrate the modification of silicon oxide microchips with a fluorophore molecule, enabling detection of reactive oxygen species via green fluorescence microscopy within living cells.

Methods

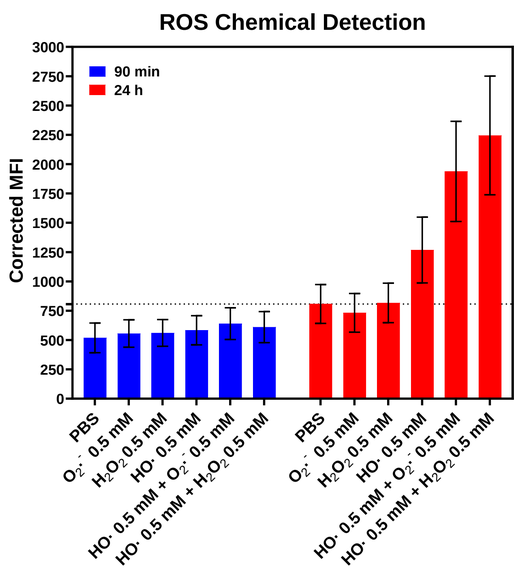
SPAchips are based on a micrometric silicon surface anchored for functionalization through surface techniques.

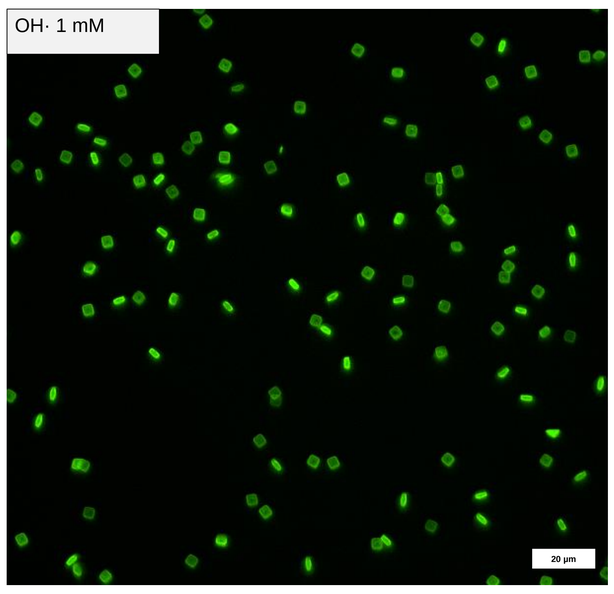
After treatment with the fluorophore, SPAchips can be detached from the surface and dispersed colloidally in suspension for cellular utilization.

ROS measurements have been generated solely following the internalization of SPAchips into the cytosol.

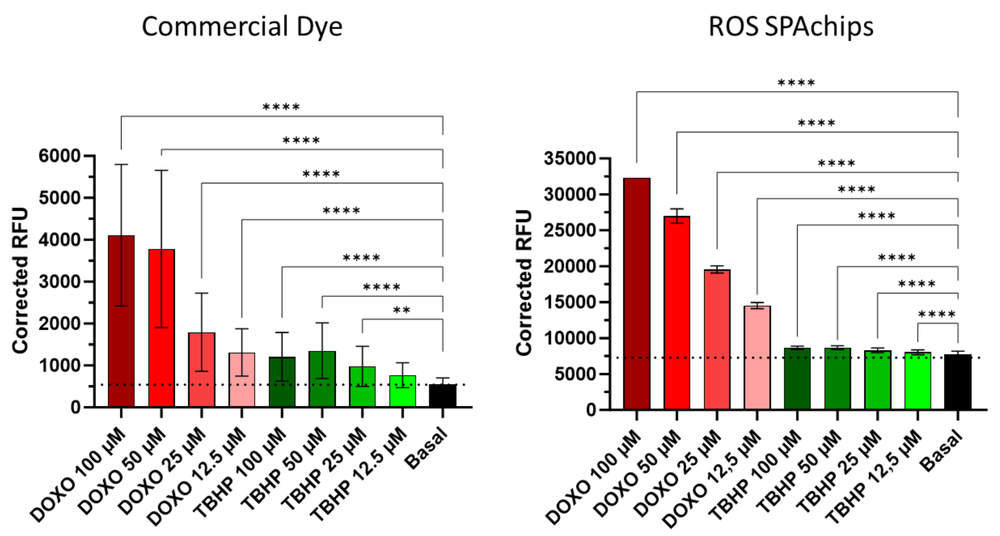


Results





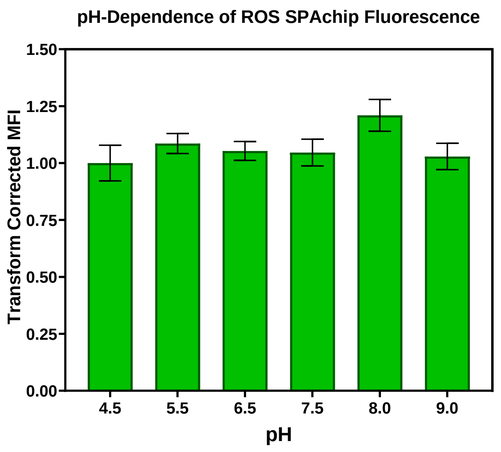
A comparative study on CCD1095sk cells after 6 hours

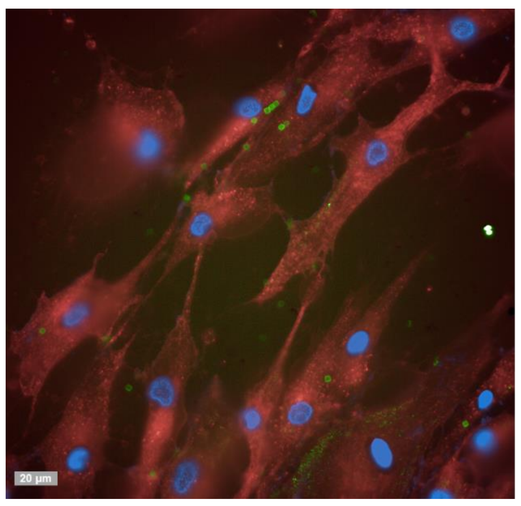


Initial results display a uniform functionalization of the molecule across the silicon surface.

ROS specificity with **OH·** is discernible following a 24-hour treatment with various species. Hydroxyl radicals are generated through the decomposition of H_2O_2 facilitated by metal ions and superoxide radicals.

SPAchips, modified with ROS fluorophores, possess the capability to measure reactive oxygen species without being affected by the pH of the solution.





In-vitro assays have been conducted to assess the internalization of ROS SPAchips® as well as their drug response against a commercial dye.

After treating the cells for 6 hours, ROS SPAchips and the dye exhibit a correlation with the concentration.

Conclusions

1. ROS SPAchips offer an innovative solution: can be modified with fluorescence molecules that react to reactive oxygen species, thereby enabling the dynamic detection inside living cells.
2. The fluorophore used to functionalise the chip surface allows the measurement of ROS produced inside the cells without response to the pH solution, improving the properties described in the main commercial ROS kits.
3. Results have shown that SPAchips internalise into different cell types without damaging living cells. Thanks to their low toxicity, ROS production in living cells can be monitored over time.

References

1. Torras, N.; Agusil, J. P.; Vázquez, P.; Duch, M.; Hernández-Pinto, A. M.; Samitier, J.; De La Rosa, E. J.; Esteve, J.; Suárez, T.; Pérez-García, L.; Plaza, J. A. Suspended Planar-Array Chips for Molecular Multiplexing at the Microscale. *Advanced Materials* **2016**, *28* (7), 1449–1454. https://doi.org/10.1002/adma.201504164

2. Espinoza, E. M.; Røise, J. J.; Li, I. C.; Das, R.; Murthy, N. Advances in Imaging Reactive Oxygen Species. *Journal of Nuclear Medicine* **2021**, *62* (4), 457–461. https://doi.org/10.2967/jnumed.120.245415.