MULTI-OMICS WORKFLOW TO CHARACTERISE OXIDATIVE STRESS AT THE MOLECULAR LEVEL USING *IN VITRO* MODELS

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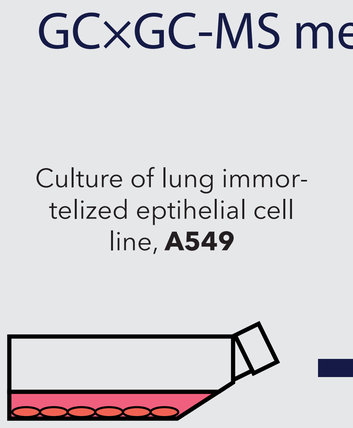
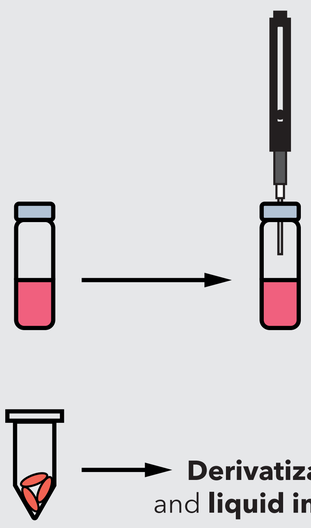
Context

Oxidative stress is a pathological condition that arises when there is an imbalance between reactive oxygen species (ROS) production and cellular detoxification ability [1]. This condition has been linked to various diseases such as asthma and cancer, making it an important area of research for better diagnosis and treatment of inflammatory diseases. *In vitro* cell cultures have become an essential tool to comprehend the intricate mechanisms of oxidative stress involved in inflammatory reactions [2]. The use of *in vitro* cell cultures provides an ethical and controlled environment where the effects of oxidative stress can be studied independently of other confounding factors. The challenge lies in establishing optimal oxidative conditions, as induced by H_2O_2, without triggering apoptosis or necrosis of the cells. However, achieving analytically reproducible conditions with biological materials is challenging. In this ongoing research, we are striving to replicate and refine a robust analytical workflow for the optimal oxidation of A549 epithelial cell lines using H_2O_2.

GC×GC-MS method

Culture of lung immortelized eptihelial cell line, **A549**

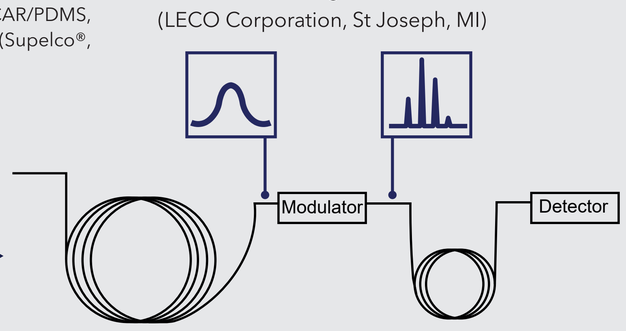




Derivatization and **liquid injection**

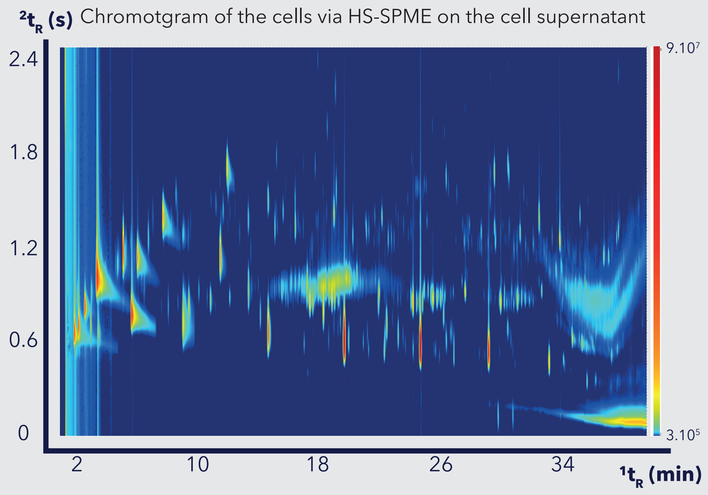
Fiber type used to trap volatile organic compounds (VOC): 50/30 µm DVB/CAR/PDMS, Stableflex core (Supelco®, ©Merck)

GC×GC-TOFMS analysis.
Instrument: Pegasus™ BT 4D (LECO Corporation, St Joseph, MI)



¹D: Non polar Rxi-5Sil MS (30m x 0.25mm x 0.25µm df)
²D: Rxi-17SilMS (1.8m x 0.25mm x 0.25µm df)
Modulation period : 2.5 s

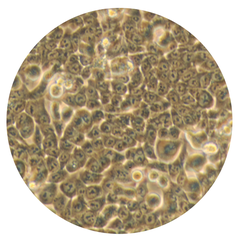
²t_R (s) Chromotgram of the cells via HS-SPME on the cell supernatant

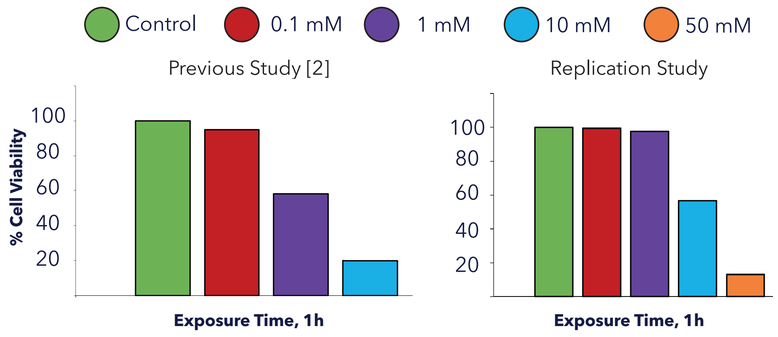


Study Workflow

1. Attempt to replicate the previous study [2]

Oxidation condition:
0.1 mM of H_2O_2 for 1 h



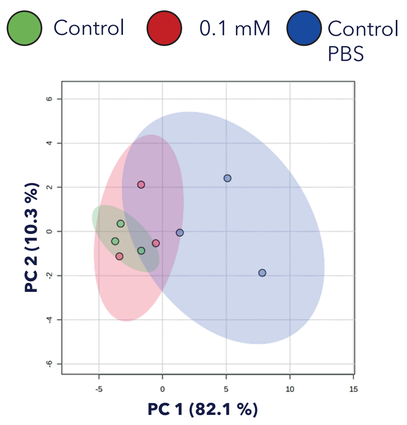


1.a Viability Test

1.b SPME analysis on the cell supernatant

1.c Results of the SPME part

Expectations	Outcomes
- Confirmation of the previous oxidation condition (0.1mM of H_2O_2 for 1h)	- The profile of the viability test is different (shifted)
- Same viability for the cell with the same oxidation conditions	- There is no clear separation accross the differents oxidations conditions
- Unambiguous clustering in the PCA for the differents conditions	- This concentration seems too «soft» to trigger any oxidation event **Are we sure about the concentration of H_2O_2?**



Solution: Titration of H_2O_2 and try to **oxidize with a higher concentration!**

Developpement of an **analytical workflow** to **ensure** the **correct H_2O_2 concentration** with a titration by $KMnO_4$ previously calibrated by a fresh solution of oxalic acid

2. Increase the concentration and set up 2 expositions times

Oxidation condition:
0.9 mM of H_2O_2 for 1 h and 2 h

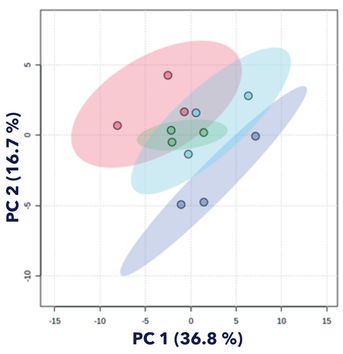
2.a Viability Test

The viability is around **98 %** for all the conditions

2.b SPME analysis on the cell supernatant

2.c Results of the SPME part

Expectations	Outcomes
- By controlling the H_2O_2 concentration, the results are more robust	- The viability of the cells is high for all the conditions, enven the 0.9 mM for 2 h
- By increasing the concentration and the exposition time, the oxidation will be triggered	- There is no clear separation accross the differents oxidations conditions
	- This concentration seems again too «soft» to trigger any oxidation event
	- A small trend can be observed between the control group (dark blue) and the rest. However, it can be linked to the **dilution** when adding H_2O_2 to the media



0.9 mM for **2 h** | 0.9 mM for **1 h** | Control | Control PBS

3. Study of the impact of the cell starving step for long exposition times

Oxidation condition:
0.1 mM of H_2O_2 for 1 h and 2 h with or without a starving step of FBS 2% for 24h

Context: Cells require serum (typically 10 % FBS/FCS) for growth and to maintain viability. However, during the oxidation process, the medium containing the oxidative agent is serum-free. Prolonged exposure, such as 24 h, would likely lead to cell death regardless of the oxidative agent used. The serum-starvation step allows the cells to adapt to a lower serum concentration, typically around 2 % for 24 h.

3.a Viability Test

The viability is around **96 %** for all the conditions

3.b SPME analysis on the cell supernatant
ONGOING

Next Step/Ongoing

- Developing an analytical derivatization workflow for the cellular pellet (utilizing metabolomic and lipidomics approaches)
  → **Comparing the VOC and approach with the derivatization/liquid approach**

- Advancing to the development of an organoid model to further enhance our understanding of the oxidation at a molecular level

[1] Forman, H. J., and Zhang, H. (2021) Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery 20*, 689–709.

[2] Zanella, D., Henket, M., Schleich, F., Dejong, T., Louis, R., Focant, J.-F., and Stefanuto, P.-H. (2020) Comparison of the effect of chemically and biologically induced inflammation on the volatile metabolite production of lung epithelial cells by GC×GC-TOFMS. *The Analyst 145*, 5148–5157.

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