TNA User Report

The completed and signed form below should be returned by email to
eurochamp2020@lisa.u-pec.fr

Project title	Online analysis of volatile organic gases released from cell cultures upon aerosol deposition as a diagnostic tool for metabolic processes
Name of the accessed chamber	PACS-C3
Number of users in the project	2
Project objectives (max 100 words)	• To optimize and characterize the analytical capability of proton-transfer-reaction mass spectrometry (PTR-MS) in measuring VOCs emitted from *in-vitro* grown human bronchial cells • To optimize the experimental set-up of a cell exposure chamber coupled to a PTR-ToF-MS instrument • To identify VOCs that are emitted from human bronchial cells upon oxidative stress caused by the application of model stressors (H_2O_2, Cu(II), 1,4-naphthoquinone) and secondary organic aerosol (SOA) • To potentially identify biomarkers of pulmonary oxidative stress that could be later used for medical purposes
Description of work (max 100 words):	• Coupling of a PTR-TOF 8000 instrument to a cell exposure chamber, optimization of the experimental design, instrument calibration • PTR-TOF measurements of VOC emissions from human bronchial cells upon oxidative stress caused by the application of model stressors (H_2O_2, Cu(II), 1,4-naphthoquinone) and SOA • Assistance in smog chamber experiments for generating SOA • PTR-ToF data analysis and quality control • Joint data interpretation with Laure-Estelle Cassagnes (PSI)

Principal Investigator's and group's information	
First name	Armin
Family name	Wisthaler
Nationality	Italian
Activity domain[1]	Chemistry
Home institution	University of Oslo
Institution legal status[2]	UNI
Email	armin.wisthaler@kjemi.uio.no
Gender	Male
User status[3]	ACA
New user	Yes

User 1 Information[4]	
First name	Liang
Family name	Zhu
Nationality	Chinese
Activity domain	Chemistry
Home institution	University of Oslo
Institution legal status	UNI
Email	liang.zhu@kjemi.uio.no
Gender	Male
User status	PDOC
New user	Yes

User 2 Information	
First name	Alexander
Family name	Håland
Nationality	Norwegian
Activity domain	Chemistry
Home institution	University of Oslo
Institution legal status	UNI
Email	alexahaa@kjemi.uio.no
Gender	Male
User status	PGR
New user	Yes

[1] Physics; Chemistry; Earth Sciences & Environment; Engineering & Technology; Mathematics; Information & Communication Technologies; Material Sciences; Energy; Social sciences; Humanities.
[2] UNI= University and Other Higher Education Organisation;
RES= Public Research Organisation (including international research organisations and private research organisations controlled by public authority);
SME= Small and Medium Enterprise;
PRV= Other Industrial and/or Profit Private Organisation;
OTH= Other type of organization.
[3] UND= Undergraduate; PGR= Post graduate; PDOC= Post-doctoral researcher; RES= Researcher ENG= Engineer; ACA= Academic; TEC= Technician.
[4] Reproduce the table for each user who accessed the infrastructure

Trans-National Access (TNA) Scientific Report

The completed and signed form below should be returned by email to eurochamp2020@lisa.u-pec.fr

Please limit the report to max 5 pages, you can include tables and figures. Please make sure to address any comments made by the reviewers at the moment of the project evaluation (if applicable, in this case you were informed beforehand). Please do not alter the layout of the document and keep it in Word version. The report will be made available on the eurochamp.org website. Should any information be confidential or not be made public, please inform us accordingly (in this case it will only be accessible by the European Commission, the EUROCHAMP-2020 project partners, and the reviewers). Please include:

- Introduction and motivation
- Scientific objectives
- Reason for choosing the simulation chamber/ calibration facility
- Method and experimental set-up
- Data description
- Preliminary results and conclusions
- Outcome and future studies
- References

Instructions

Name of the PI:	**Armin Wisthaler**
Chamber name and location:	**PACS-C3, Paul Scherrer Institut (PSI), Villigen, Switzerland**
Campaign name and period:	**Online analysis of volatile organic gases released from cell cultures upon aerosol deposition as a diagnostic tool for metabolic processes 12/02/2018 – 16/02/2018; 05/03/2018 – 09/03/2018**

Introduction and motivation

Air pollution has been recently estimated to cause about 4.3 million deaths per year and 123 million year of life lost.[5] Particulate matter contains chemical species such as transition metals (Fe, Cu, Mn…), quinones, organic peroxides and elemental carbon that may lead to oxidative stress at the inner surface of the lungs. However, the link between the chemical composition of particulate matter (PM) and the adverse health outcome in humans remains unclear and there is a need to develop pulmonary oxidative stress biomarkers that could be used as measures for impaired health by air pollution in medical practice or in epidemiological studies.

Many volatile organic compounds (VOCs) are produced in the human body and emitted through exhalation or excretion through the skin, in the feces and in urine. The elaboration of specific marker compounds or metabolic profiles in the human breath as indicator of diseases has become a hot research topic in medical diagnostics. Proton-transfer-reaction mass spectrometry (PTR-MS) is widely used for clinical analysis of gas exhaled by humans, since it is a fast, direct and non-invasive method.[6]

[5] Lelieved J. Clean air in the Anthropocene. Faraday Discuss. 2017;(200), 693-703

[6] Herbig J et al. Proton transfer reaction-mass spectrometry applications in medical research. J. Breath Res. 2009;3(2):020201

The literature regarding the use of PTR-MS for characterizing emissions from cell cultures is, however, relatively scarce and not related to air pollution toxicity. We thus used a state-of-the-art high resolution proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) instrument for investigating emissions of VOCs from human bronchial cells that were exposed to model stressors and secondary organic aerosols (SOA).

Scientific objectives

The scientific objectives of our exploratory study were

i) To optimize and characterize the analytical capability of PTR-MS in measuring VOCs emitted from *in-vitro* grown human bronchial cells

ii) To optimize the experimental design of a cell exposure chamber coupled to a PTR-ToF-MS instrument

iii) To identify VOCs that are emitted from human bronchial cells upon oxidative stress caused by the application of model stressors (H_2O_2, Cu(II), 1,4-naphthoquinone) and SOA

iv) To potentially identify biomarkers of pulmonary oxidative stress that could be later used for medical purposes

Reason for choosing the simulation chamber/ calibration facility

PSI hosts unique research infrastructure which includes three atmosphere simulation chambers (PACS-C3) and a small cell exposure chamber. In addition, PSI has a well-established research collaboration with an air toxicology group[7] which is a necessary prerequisite for conducting front-end-research at the interface between atmospheric chemistry and life sciences.

Method and experimental set-up

Figure 1 The cell exposure chamber connected to the PTR-TOF 8000 sampling tube (black hose)

The cell exposure chamber used at PSI has been described in detail previously.[8] The chamber was coupled to a PTR-ToF-MS instrument (PTR-TOF 8000; Ionicon Analytik, Innsbruck, Austria) provided by the University of Oslo (Figure 1). The flow through the cell exposure chamber was adjusted to increase the concentration of VOCs in the dynamic headspace of the cells. Human bronchial epithelial cells (BEAS-2B) were grown on inserts, rinsed with Dulbecco's Phosphate Buffered Saline (DPBS) before the experiments, placed in the exposure chamber and subjected to different compounds inducing oxidative stress.

In a first set of experiments, cells were exposed to model stressors including hydrogen peroxide (H_2O_2), copper (Cu(II)), 1,4-naphthoquinone (1,4-NQ) and a combination of Cu(II) and 1,4-NQ. The compounds were injected in aqueous solution at the air-liquid interface (ALI) of the cell cultures and thereafter the profile of VOC emission was recorded for

[7] Group Geiser, Institute of Anatomy, University of Bern http://www.ana.unibe.ch/forschung/gruppe_geiser/index_ger.html

[8] Mertes, P.; Praplan, A.P.; Künzi, L.; Dommen, J.; Baltensperger, U.; Geiser, M.; Weingartner, E.; Ricka, J.; Fierz, M.; Kalberer, M. A compact and portable deposition chamber to study nanoparticles in air-exposed tissue. J. Aerosol Med. Pulm. Drug Deliv. 2013, 26, 228–235.

at least 3 hours by PTR-ToF-MS. Two types of blank experiments were carried out: the cellular blank (water only onto cells) and the acellular blank (test compound in aqueous solution onto DPBS).

In a second set of experiments, cells were exposed to filter extracts from primary organic aerosol (POA) and SOA derived from wood burning. We also conducted α-pinene ozonoloysis experiments in one of the PACS-C3 atmosphere simulation chambers. Fresh and aged (~1 d) SOA was collected onto quartz filters, extracted with Milli-Q water and injected onto the cells. In addition to the VOC measurements, the cytotoxicity of the applied compounds was assessed with the Lactate Dehydrogenase (LDH) assay.

Data description

We have generated quality-assured PTR-ToF-MS data (*m/z* vs. *time*) that describe emissions of VOCs from human bronchial cells subjected to different stressors (H_2O_2, Cu(II), 1,4-NQ, wood-burning-derived POA and SOA, fresh and aged SOA from α-pinene ozonoloysis). The data set includes a series of control experiments (cellular blank, acellular blank). Data analysis and interpretation is ongoing.

Preliminary results and conclusions

A low and stable mass spectral background was observed following a short period of stabilization after closure of the cell chamber. A series of test compounds (Table 1) were injected at different concentrations onto a monolayer of BEAS-2B cells at the ALI.

Table 1. List of the different compounds injected onto DPBS or BEAS-2B cells.

Compounds tested	Concentration in µg/cm^2 of cell surface	
H_2O_2	100	--
1,4-naphthoquinone	2	0.2
Cu(II)	0.2	0.02
1,4-naphthoquinone + Cu(II)	2 + 0.2	--
Wood burning POA	1	0.1
Wood burning SOA	1	0.1
α-pinene SOA (fresh)	1	0.1
α-pinene SOA (aged)	1	0.1

H_2O_2 at high concentration (100 µg/cm^2) was used as a positive control for reactive oxygen species (ROS) induced cytotoxicity as we observed a 50% cell death at this concentration.

In the first set of experiments, the cells were treated with H_2O_2, 1,4-NQ and Cu(II) alone or combined at different concentrations. As expected, injection of copper onto DPBS or onto the cells did not induce any VOC emissions, nor did it induce any specific cytotoxicity. Upon injection of 1,4-NQ, a sharp increase of *m/z* 159.04 (corresponding to protonated 1,4-NQ) was observed, both with cells present and in the acellular blank. The signal decreased much faster with the cells being present, suggesting a potential adsorption onto the cells or internalization/metabolization by the cells. This process was not affected by the addition of copper to the mixture. The combined injection of 1,4-NQ and Cu(II) triggered the emission of VOCs that were not observed in the DPBS control experiments or upon injection of a single compound. The observed ions remain to be assigned to specific neutral precursors and linked to specific biological mechanisms. The obtained results may give some hints on the biological mechanism triggered by redox cycling.

In a second set of experiments, the cells were treated with filter extracts from chamber studies in the PACS-C3 facility. α-pinene ozonolysis experiments were performed just before (< 30 min) injection onto the cells, meaning that we were able to work with very fresh samples. SOA was also allowed to age for ~ 1 day on the filter before being extracted and injected onto the cells. Additional

experiments were carried out with wood burning POA and SOA filter extracts. The *m/z* signals observed in the aerosol exposure experiments differed from those observed in the model stressor experiments and showed a dose-response relationship. Exposure to POA *vs.* SOA and fresh SOA *vs.* aged SOA generated different signal intensities. One specific *m/z* signal increased in all experiments suggesting that it stems from a general marker of cellular stress. All data need to be subjected to further analysis before being released in more detail.

We conclude that our innovative setup combining the PACS-C3 facility, a cell exposure chamber and a PTR-ToF-MS instrument is suitable for investigating the release of VOCs from cell cultures upon aerosol deposition and has given promising preliminary results. The set-up at PSI allows to inject very fresh samples onto the cells, thus reducing the decay of reactive oxygen species in the aerosol. The low limit of detection and high mass resolution of the PTR-ToF-MS instrument allow to detect ppt levels of emitted VOCs and to identify their elemental composition. The online and real-time capability of the instrument allows to monitor the kinetic profiles of emitted VOCs, which are typically released within one hour after the injection of the stressors.

Outcome and future studies

A PTR-TOF 8000 instrument from the University of Oslo was successfully coupled to a cell exposure chamber at PSI. It was used for monitoring of VOC emissions from human bronchial cells upon oxidative stress caused by the application of model stressors (H_2O_2, Cu(II), 1,4-NQ) and different types of aerosols. Further data analysis is needed to differentiate between VOCs originating from the compounds injected or from the cells as a reaction to the treatment. Further work is required for identifying the biological mechanism linked to the observed cellular VOC release. Biological assay studies on the metabolism of quinone and on SOA toxicity will be performed at PSI to link VOC emissions to biological response mechanisms.