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Airway epithelium co-cultured with immune cells for a better assessment of the low dose effects of environmental pollutants

Emilie RICQUEBOURG , Gabrielle VERNOUILLET, Isabelle MOMAS, Nathalie SETA, Sophie ACHARD

Public Health and Environment Laboratory, EA4064 - Paris Descartes University

PURPOSE

People are daily exposed to various pollutants present in airborne pollution. Many epidemiological studies revealed the existence of an association between indoor air pollution (aldehydes, VOCs, …) and the respiratory disorders. To study a such relationship, it is important to have toxicological elements to clarify the impact of these pollutants on cellular or tissue targets. Development of new *in vitro* models to assess the impact of environmental pollutants on human health is necessary.

The present study investigated the pertinence to use a reconstituted epithelium, a 3D-model close to the human respiratory epithelium, co-cultured with immune cells, to assess the impact of environmental pollutants, such as volatile organic compounds (VOC), on biological activity.

ASSESS THE IMPACT OF REPEATED EXPOSURES TO VOC, EMITTED BY PAINTS, ON THE CELLULAR RESPONSES OF THE HUMAN RECONSTITUTED NASAL EPITHELIUM (MUCI) CO-CULTURED, OR NOT, WITH IMMUNE CELLS (IC)

RESULTS - DISCUSSION

3 experimental conditions were considered :

- "Control" : tissue-control maintained in the incubator (static exposure)
- "Air" : tissue-control exposed to air-flow (dynamic exposure ; 2mL/min)
- "VOC" : tissue exposed to an atmosphere charged with VOC (dynamic exposure ; 2mL/min)

Morphological changes were observed when epithelium was co-cultured with macrophage (Muci-IC) in comparison with epithelium alone (Muci) : epithelium thickness increased by 35% in presence of immune cells (Muci-IC *versus* Muci) whatever the experimental conditions.
This observation was correlated with basal cell proliferation *(Ki67 immunostaining)*.

Dynamic Air-exposure (Air) had no effect on the epithelium thickness compared to static exposure (Control) whatever the 3D-model.

VOC-exposure (VOC) did not affect the epithelium thickness in comparison to Air-exposure (Air) whatever the 3D-model. A trend of mucus production increase appeared (Alcian blue staining).

Tissue integrity, assessed by *TEER measurements*, was preserved *(data not shown)* for all experimental conditions until 3 weeks.

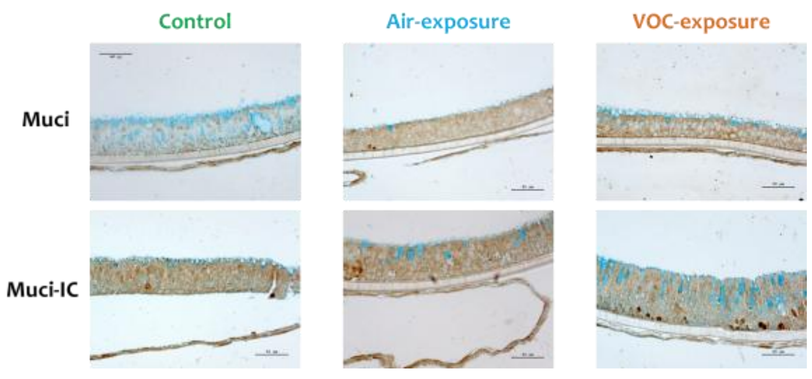

Fig. 1 : Epithelium cross-sections after 2 weeks of experiments (magnification x400)

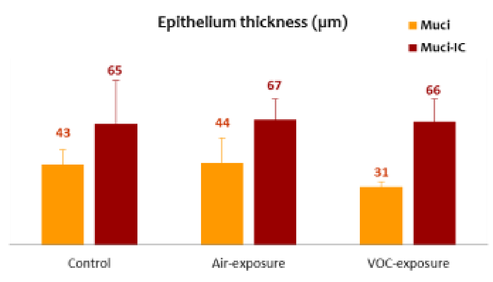

Fig. 2 : Epithelium thickness evaluation

In addition to morphological change , inflammatory response was observed by an increase of IL-8 production :

- after Air-exposure compared to "Control" what showed a positive impact of the Air-flow (2mL/min) on the cytokine production.
- after VOC-exposure compared to Air-exposure, only for co-culture Muci-IC. These results indicated an increased sensitization of nasal epithelium exposed to VOC due to IC presence.

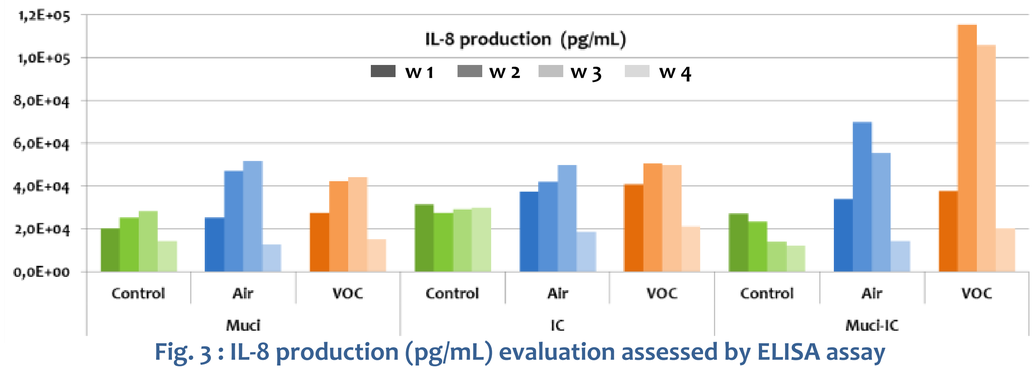

Fig. 3 : IL-8 production (pg/mL) evaluation assessed by ELISA assay

OUR RESULTS SHOWED A POSITIVE IMPACT ON TISSUE PROLIFERATION AND AN INCREASE OF HUMAN RECONSTITUTED NASAL EPITHELIUM SENSITIZATION TO ENVIRONMENTAL POLLUTANTS WHEN THIS 3D-MODEL WAS CO-CULTURED WITH IMMUNE CELLS

METHODS

MUCI-IC CO-CULTURE MODEL

- **Muci :** A human reconstituted nasal epithelium[1] cultured under air-liquid conditions **on 0.33 cm² porous membrane** (MucilAir™ Epithelix, Switzerland).
- **IC :** IC were cultured under submerged conditions **on 1.12 cm² porous membrane**, after THP-1 differentiation with phorbol myristate acetate into macrophage-like cells[2] (IC) (Fig 4).
- **Muci-IC co-culture :** Muci and IC were superposed as shown on Fig 5.

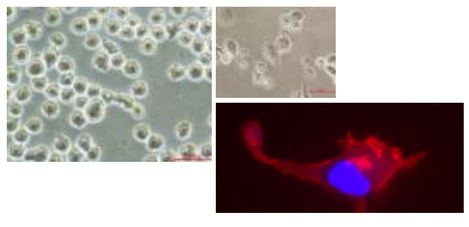

Fig 4 : THP1 differentiated into macrophage-like cells

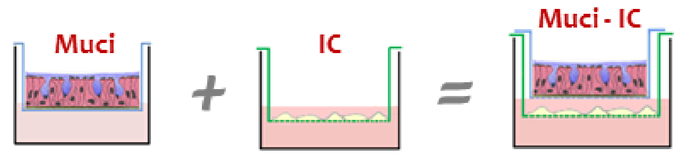

Fig 5 : Muci-IC co-culture design

VOC GENERATION AND REPEATED EXPOSURES

1- **Air** generated using a compressor
2- Flow meter to fix the **air renewal** at **25%**
3- **Humidification Chamber** (Vitrocell® System, Germany)
4- Amount of aqueous **domestic-paint :** 1.16g on 150cm²
(NF EN ISO 16000-9 et NF EN ISO 16000-11)
5- **Control of the VOC mixture** : passive sampling using SPME fiber and GC/MS analysis.
6- **Repeated exposures**

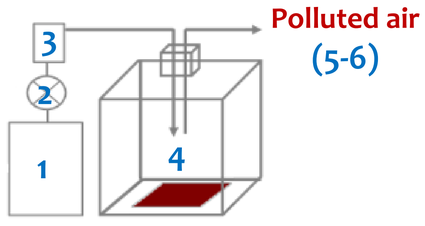

Fig 6 : VOC generation chamber

Our system was adapted from the guidelines concerning the emission of VOC in indoor air. It consists in a glass chamber (11L) containing a painted surface (4). 25% of the air (1-3) in the chamber is renewed every hour (2) (Fig.6).

After 3 days of VOC emission, the generated atmosphere was controlled[3] (5) and epithelia were exposed for 1 hour, once a week during 4 weeks (6). Repeated exposures were applied with a flow of 2mL/min at the air-liquid interface (Fig.7-8).

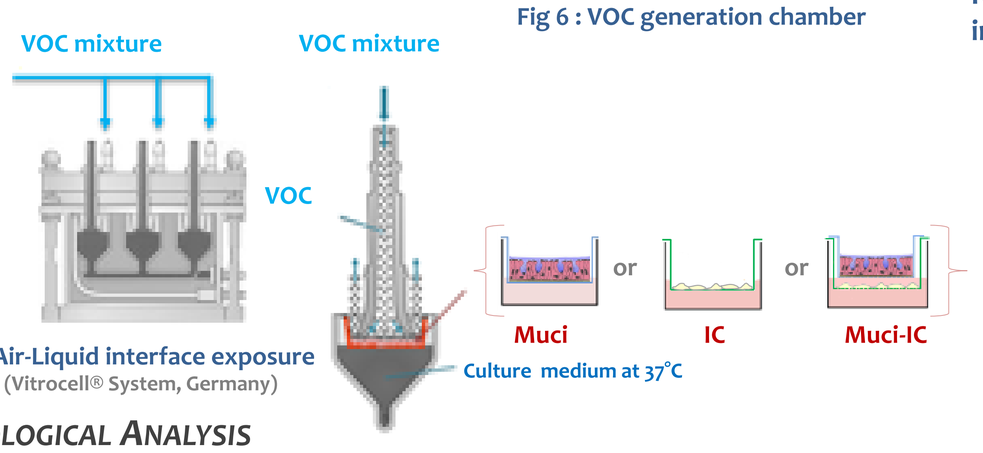

Fig 7 : Air-Liquid interface exposure (Vitrocell® System, Germany)

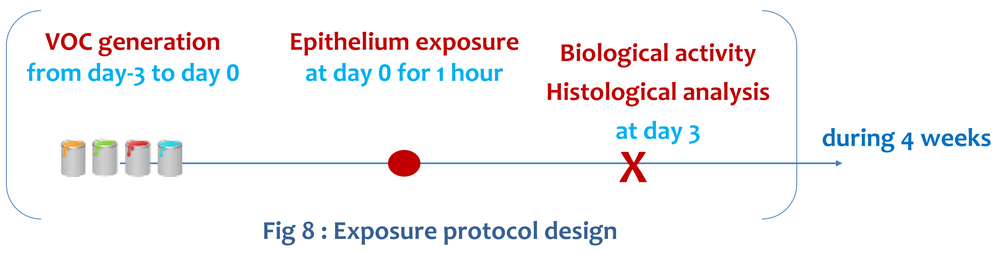

Fig 8 : Exposure protocol design

BIOLOGICAL ACTIVITY AND HISTOLOGICAL ANALYSIS

72h after exposure:

- Culture media were collected and cytokine production (IL-8) were evaluated by ELISA assay (R&D system).
- Epithelia were scarified, fixed in 4% paraformaldehyde, embedded to paraffin to perform 4µm-thick sections. Then cross-sections were stained using Ki-67 antibody (Abcam) coupled to peroxydase and Alcian blue (Sigma).

1- *Huang et al, 2009 ;* 2- *Puig-Kröger et al, 2004 ;* 3- *Bourdin et al, 2014*

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