In vitro Models to Identify Respiratory Sensitizers -An Update

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 - Mouse versus human
 - in vivo versus in vitro "anatomy"
- Air-liquid Interphase (ALI) culture
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- GARDAir (Senzagen)
- "VitralizeMe" (LIST)
- Outlook

Pulmonary models

From simple models to in vivo models

Available models

- in vitro
- in vivo
- ex vivo

Available exposure systems

- Submerged exposure
- ALI exposure + aerosol nebulizer
- ALI exposure + particle sprayer
- Intra-tracheal instillation
- In vivo inhalation
- Ex vivo perfusion



AIR LIQUID INTERFACE

Medium/Low cost

- Medium/High throughput
- Physiologically relevant
- Disease relevant

Mouse versus Human Airways



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Current organisation of the *in In vivo* anatomy of the *vitro* system alveoli



Why Air - Liquid Interphase

? in vitro – in vivo ?





Lacroix et al., 2018 AIVT http://socratic.org/questions/how-does-the-structure-of-the-alveoli-relate-to-its-function-in-the-lungs

Characterization of the 3D in vitro model: structure and functionality



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Klein et al., PFT 2013

SURFACTANT PRODUCTION IN A549 CELLS

Surfactant protein A

Surfactant protein B

Surfactant protein C



SURFACTANT ON ALVEOLAR TYPE II CELLS





Alveolar type II cells



Endothelial cells

Characterization of the 3D in vitro model: structure and functionality



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Current standard of testing?

in vivo model



LLNA assay

EthicsCostsRegulation

in vitro model

Current standard of testing

Models for skin sensitization





LLNA





GARD assay

"VitralizeMe"

Model for chemical irritants/sensitizers



AIM: Develop an *in vitro* model which mimic at best the alveolar barrier to assess the respiratory sensitization potential of different compounds



- Dendritic-like cells (THP-1)
- Endothelial cells (EA.hy 926)
- : Alveolar type II epithelial cells (A549)

 Macrophages (THP-1 differentiated with PMA)

- : Coculture medium
- : Surfactant
- $1_{ }$: Transwell insert

Alveolar macrophages

- Engulf and remove foreign materials by phagocytosis (Sibille and Reynolds, 1990)
- Regulate modulation/suppression DCs activation by IL-10 and/or IL-12 release (Bedoret *et al.*, 2009; Lauzon-Joset *et al.*, 2014; Toussaint *et al.*, 2013).



- Sampling, recognition and processing of antigens by dendrites extension through the epithelial barrier (*Holt et al., 1994;* Gills *al.*, 2012)
- Upon activation up-regulate adhesion and co-stimulatory molecules such as CD54 CD40, CD 80, CD 86, OX40L involved in different phases of immune response (Cumberbatch *et al.*, 1997, Ito 2012, Besnard 2011)



Chary et al., 2019; WO2018/122219 A1

The GARD platform - biological system





GARDair - Identification of chemical

respiratory sensitizers

EU Commission – Flagship Product Health :

"GARDair – The first predictive in vitro assay for the identification of respiratory sensitizers."

€2.4 M in funding



This project has recieved funding from the European Union's *Horizon* 2020 Research and Innovation Programme under grant agreement No 756014.



GARDair - Identification of chemical

respiratory sensitizers



GARDair - coverage of mechanistically

relevant pathways



SENZA GEN

GARDair - in-house validation

8 Performance



Test substance with mean GARD decision value from biological triplicate samples > 0 = Respiratory Sensitizer





Case study: chemical irritants / sensitizers INSTITUTE OF SCIENCE

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Selected working concentrations to have around 90% of cell viability







Acr = Acrolein → respiratory irritant PA = Phthallic anhydryde → respiratory sensitizer TMA = Trimellitic acid → respiratory sensitizer

Cytokines pattern





Comparative pattern of effects

| | Acrolein | | PA | | TMA | |
|--------|----------|-----|-----|-----|-----|-----|
| | 24h | 48h | 24h | 48h | 24h | 48h |
| MCP-1 | К | = | = | 7 | = | = |
| MIP-3a | К | Ъ | 7 | 7 | 7 | 7 |
| IL-6 | К | Ъ | 7 | 7 | 7 | 7 |
| IL-7 | 7 | 7 | = | = | = | = |
| RANTES | = | 7 | 7 | 7 | 7 | 7 |
| GM-CSF | К | Ъ | 7 | 7 | 7 | 7 |
| IL-10 | К | Ы | 7 | 7 | 7 | = |
| | | | | | | |

- Definition of respiratory irritation/sensitization markers
- Panel of cytokines able to identify respiratory sensitizers
- Possibility to discriminate between respiratory irritants and sensitizers

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WO2018/122219 A1

Unique set of 11 endpoints



| | | Chemical | Chemical | Protein |
|----------------------|---------|------------|----------|-------------|
| | | sensitizer | irritant | sensitizers |
| Cell surface markers | | | | |
| | CD54 | 7 | = | = |
| | TSLPr | 7 | = | = |
| | OX40L | = | N | 7 |
| Cytokines i | release | | | |
| | IL6 | 7 | = | = |
| | IL7 | = | 7 | = |
| | IL10 | 7 | = | = |
| | MCP-1 | 7 | Ы | = |
| | GM-CSF | 7 | Ы | = |
| Gene expre | ession | | | |
| | IL1R1-1 | 7 | N | Ы |
| | CIITA | Ы | 7 | = |
| | HLA-DRA | = | 7 | = |





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The evolution of the 3D tetra-culture model at "LIST"



2008: Tetraculture 6-w format

Fonds National de la

Recherche Luxemboura

Epithelial cells, mast cells and immuno cells located on the bottom of the well, endothelial cells on the apical side of a Transwell[™] insert. Apical cells cultured in SUBMERGED conditions. 2011: Tetraculture 6-w format Epithelial cells, mast cells and immuno cells located on the apical side of a TranswellTM insert; endothelial cells seeded on the bottom of the well. Apical cells cultured at ALI conditions. 2013: Tetraculture 6-w format Epithelial cells, mast cells and immuno cells located on the apical side of a Transwell[™] insert; endothelial cells seeded on the basolateral side of the Transwell[™] insert. Apical cells cultured at ALI conditions. 2017: Tetraculture 6-w format Epithelial cells and immuno cells located on the apical side of a Transwell[™] insert; endothelial cells seeded on the basolateral side of the Transwell[™] Insert. DC cells on the basolateral side. Apical cells cultured at ALI conditions.



2017: Downscaled triculture (24-w format) Epithelial cells and immuno cells located on the apical side of a Transwell[™] insert; endothelial cells seeded on the basolateral side of the Transwell[™] insert. Reduced number of immune cells Improved shelf life

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Shippability

Apical cells cultured at ALI conditions.

HORIZON 2020

Moschini et al., 2017 (LIST)

VITROCELL[™] CLOUD CHAMBER



https://www.vitrocell.com/Portals/0/videos/vitrocell-cloud.mp4

Current standard of testing?



Who we are





https://www.google.lu/search?q=lung+puzzle&source=lnms&tbm=isch&sa=X&ved=0ahUKEwje64_xgKrWAhWjC5oKHS4TBkkQ_AUICigB&biw=1536&bih=759#imgrc=SdwDsueAcA3c4M:



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