Human-derived, state-of-the-art Lung Models to Evaluate Exposure-induced Adverse Events that can Contribute to Pulmonary Disease

Holger P. Behrsing, Ph.D.
Principal Scientist
Respiratory Toxicology Program
Presentation Outline:

1. Respiratory Toxicology at IIVS
2. Brief Revisit of the Respiratory Tract
   – Airway tissue transitions and Deposition of materials
3. *In vitro/ex vivo* Models (3D)
   – Reconstructed human airways (RHuA)
   – Precision-cut lung slices (PCLS)
4. Exposure Methods: Conventional vs high tech
5. Choice of systems: Screening vs high content
6. OPPTS 870.1300 Acute Inhalation Toxicity
   – Acute inhalation toxicity alternatives: what *can* we do now?
IIVS’s Three-pronged Approach

Practical Knowledge

*(Science)*

Dissemination of Information

*(Education)*

Advocacy for the Methods

*(Outreach)*

Increased Use and Regulatory Acceptance …of *in vitro/ex vivo* Respiratory Toxicology

**Workshop series:**

1. “In Vitro COPD Models for Tobacco Regulatory Science”
   

   Ongoing, multi-lab effort...


Respiratory Toxicology: IIVS

Assessment of inhaled (& other) materials:
- Occupational exposures (e.g. nanoparticles)
- Household products (e.g. cleaning agents)
- Personal care products (e.g. talcs)
- Fragrances (e.g. perfumes, air fresheners)
- Next Gen Tobacco (e.g. E-cigarette aerosols)
- Pharmaceuticals in development

http://www.familygonegreen.com/2014/03/02/clean-sanitize-home-without-harsh-chemicals/
http://hbgurus.com/the-blog/
http://healthimpactnews.com/2014/heavenly-scents-or-toxic-fumes-are-your-fragrances-healing-or-killing-you/
http://www.klocke.com/uploads/images/Analytik%204.jpg
Brief Revisit of the Respiratory Tract

- Airway tissue transitions: Cell types & functionality change... ...as does exposure!

Deposition of materials:
particle size and distribution along respiratory tract differs substantially

Deposition mechanisms:
- diffusion
- sedimentation
- inertial impaction
- interception
- electrostatic forces

_all play a major or minor role_
Adverse Respiratory Events: Choice of Test System

**Predictive Tools**
- Non-cellular test systems
- Databases
- Known chemistries

**Initiating event:**
**Tobacco exposure or other toxic insult to lung epithelium**

**E.g.**
- Oxidative stress
- Initiation of signaling pathways

**In silico**

**Initiating event:**
**Tobacco exposure or other toxic insult to lung epithelium**

**E.g.**
- Oxidative stress
- Initiation of signaling pathways

**In chemico**

**Tissue Response:**
- Cytokines & chemokines
- Inflammation
- Monocyte recruitment
- Protease/antiprotease imbalance

**In vitro (2D):** Reporter lines, primary cells

**In vitro (3D):** Reconstructed human tissues (organoids, spheroids, etc.)

**Ex vivo (3D):** Whole lungs, precision-cut lung slices (PCLS)

**In silico**

**In chemico**

**Tissue Effects:**
- GCH
- Impaired mucociliary clearance
- Tissue destruction
- Collagen deposition

**Pulmonary Effects:**
- Tissue remodeling
- Chronic inflammation
- Fibrosis
- Reduced airflow
- Hyperinflation

**Lung Disease/COPD:**
- Chronic bronchitis
- Emphysema
- Small airways disease
- Increased susceptibility to infection and air pollutants

**Increasing cost and/or complexity**

**Biological systems: Progression of Adverse Events following Exposure**
Respiratory Tract Models: Reconstructed Tissues

https://en.wikipedia.org/wiki/Respiratory_tract

MatTek’s EpiAlveolar™

Epithelix’s SmallAir™

http://www.epithelix.com/products/smallair

Brown CC10(+) = Club cells

Epithelix’s SmallAir™

MatTek’s EpiAlveolar™

https://pbs.twimg.com/media/BoA-xHmCcAA5ODQ.png:large
Reconstructed Human Airways (RHuA)

(e.g. MatTek’s EpiAirway™ or Epithelix’s MucilAir™)

**Compartments**

- **Apical Rinse (lavage fluid)**
  - Inhalation exposures
  - Surfactant changes
  - Leakage/signaling marker responses (LDH, cytokines, chemokines)

- **Airway (tissue)**
  - Tissue responses (multicellular)
  - -omics, biomarker regulation
  - Histology – specialty stains, morphology changes

- **Medium (blood)**
  - Systemic exposures
  - Leakage/signaling marker responses (LDH, cytokines, chemokines)

**Cells:**
- Ciliated
- Goblet
- Basal

**Tissue insert at air liquid interface (ALI)**

**Markers following Apical Challenge: Where are they?**

- All compartments sampled for marker presence/expression

### IL-6

<table>
<thead>
<tr>
<th>Condition</th>
<th>Medium</th>
<th>Lysate</th>
<th>Apical Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (-) Control</td>
<td>19</td>
<td>228</td>
<td>206</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>100</td>
<td>100</td>
<td>279</td>
</tr>
<tr>
<td>LPS</td>
<td>215</td>
<td>38</td>
<td>121</td>
</tr>
</tbody>
</table>

### IL-8

<table>
<thead>
<tr>
<th>Condition</th>
<th>Medium</th>
<th>Lysate</th>
<th>Apical Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (-) Control</td>
<td>277</td>
<td>227</td>
<td>85</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>100</td>
<td>100</td>
<td>191</td>
</tr>
<tr>
<td>LPS</td>
<td>1,049</td>
<td>632</td>
<td>176</td>
</tr>
</tbody>
</table>

### IP-10

<table>
<thead>
<tr>
<th>Condition</th>
<th>Medium</th>
<th>Lysate</th>
<th>Apical Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (-) Control</td>
<td>198</td>
<td>100</td>
<td>2,503</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>16,041</td>
<td>13,897</td>
<td>102</td>
</tr>
<tr>
<td>LPS</td>
<td>2,802</td>
<td>102</td>
<td>102</td>
</tr>
</tbody>
</table>
Reconstructed Human Airways: Mucociliary clearance

Ciliary Beat Frequency (SAVA system)

10 uM procaterol increases CBF

...but not if IL-13 treated

Goblet Cell Hyperplasia (day 14)

MUC5AC staining

Control 10 ng/mL IL-13
In Vitro/Ex vivo Models: PCLS

PCLS can represent all lung regions present in tissue source

http://intranet.tdmu.edu.ua/data/kafedra/internal/anatomy/classes_stud/en/nurse/1/adn/ptn/1/13.%20NASAL%20CAVITY,%20LARYNX.htm
HuPCLS: Long Term Culture

Untreated Control: Day 0

Untreated Control: Day 16

Rat PCLS: 10 mU/ml bleomycin D8

Rat PCLS: 100 μM BCNU D28
Particle Exposures to HuPCLS

Active Macrophages at Day 16

500 µg/mL Min-U-sil 5 (quartz silica) at Day 16

Pathologist:
• Macrophages engulfing particles
• But, are these silica? Look carbon based
• May have been donor inhaled?

Pathologist:
• Particles seemingly inert
• No macrophages engulfing
• No overt toxic events seen
500 µg/mL Min-U-sil 5 Exposure to HuPCLS

WST-8 Viability

No cytotoxicity

Note: some cytokine values were above the standard curve and values extrapolated by the xPonent software
Exposure Methods: Solubles & Aerosols

Aqueous and DMSO solubilized material, applied to apical surface

• Repeat exposures?
• Hypoxia?
• Relevance?

Apical Volume Delivery

http://www.ibric.org/myboard/print.php?id=79710&Board=new_protech

Smoke/Aerosol /Gas Delivery

http://www.vitrocell.com/inhalation-toxicology/method

TECAN/HP D300

Novel: Digital Dispensing

VITROCELL® VC-1

• Hybrid technology?
• DMSO or aqueous-based solutions
• Minimal impact on apical rheology

• Most relevant!
• Dosimetry?
• Cost!

Accurate delivery to ALI
• Client requires evaluation of pulmonary risk
• Study focus is on respiratory changes/function endpoints
• Study design can be acute or chronic exposure

3D Cell systems:
1. Organoids/spheroids
2. Reconstructed Human Airways
3. Human Precision-cut Lung Slices

Mucociliary Clearance
- Mucus production, Goblet cell hyperplasia
- Ciliary Beat Frequency

Oxidative Stress
- GSH:GSSG levels
- ARE activity

Cytotoxicity
- LDH/AK release
- Viability loss (MTT/WST-8)

Inflammation
- Cytokines
- NFκB activity
- Activated macrophages

Tissue/structural changes
- Collagen deposition
- Airway vs alveolar toxicity
Acute inhalation toxicity: adverse effect caused by substance (≤ 24 hr exposure)

Important Factors: concentration, size, shape, density (must be capable of being inhaled)

Approaches:
1. Use data from substantially similar materials/mixtures
2. Limit test: 5 males & 5 females exposed to 2 mg/L for 4hr (or highest attainable lower dose)...if no lethality, no further testing is needed
3. Conventional acute tox testing: Several groups of animals, graduated exposure concentrations, observations of effects and death are made. Factors to monitor: air flow during exposure, particle size, temperature. Necropsy findings, LC50 values, and detailed observations noted are reviewed

Alternative Approaches?:
1. Can test materials on several human donor tissues (RHuA, organoids/spheroids, PCLS)
2. State-of-the-art inhalation exposure systems for controlled air flow during exposure. Particle sizes can be measured, studies temperature-controlled
   • Detailed histology on: human airway cross sections (RHuA), native human deep lung architecture (PCLS)
Acknowledgements

- IIVS’s staff, supporters & collaborators!

Questions?

www.iivs.org

Holger Behrsing, Ph.D.: hbehrsing@iivs.org