In Vitro Models Available for Testing of ENDS

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Outline:

1. In Vitro Toxicology Approach at IIVS
   1. Science, education, & outreach
   2. Impact of accepted methods
2. Adverse Respiratory Events: *in vitro/ex vivo* systems
   1. Utility of 2D, focus on 3D
3. E-cigarette exposures & examples of models
   1. Occupational, inadvertent, and **vaping**
4. Pragmatic use of *in vitro/ex vivo* models
   1. Reconstructed human airway tissue
   2. Precision-cut lung slices (PCLS)
5. Exposure systems
6. Opportunity for *in vitro/ex vivo* non-animal methods
IIVS (non-profit): 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* Assays and Methods for product and safety evaluation
   • FDA R13 Small Conference Grant Support!


3. “In Vitro Exposure Systems and Dosimetry Assessment Tools for Inhaled Tobacco Products” April 4-6, 2016; Bethesda, MD

4. TBD...
In Vitro Regulatory Acceptance Helps us All

• IIVS participated in:
  – ECVAM-sponsored validation studies (corrosivity, eye irritation, skin irritation, phototoxicity) & retrospective evaluations (eye irritation)
  – ICCVAM validations or method evaluations (acute systemic toxicity, severe eye irritants)
  – OECD expert groups to develop in vitro Test Guidelines (skin corrosivity, severe eye irritants, 3T3 NRU phototoxicity, skin irritation, cell transformation assays, skin sensitization assays)

• What does a standardized/validated assay provide?
  – A common mechanism available to the industry to generate data that has credibility, with ongoing data collection feeding reference databases
  – Credible data = weight of evidence that Regulators can use for decision making processes
  – A system which benefits a wide range of industries for product development and regulatory submissions
### Adverse Respiratory Events & Choice of System

#### Biological systems: Progression of Adverse Events following Exposure

<table>
<thead>
<tr>
<th>Predictive Tools</th>
<th>Initiating event: Tobacco exposure or other toxic insult to lung epithelium</th>
<th>Tissue Response: E.g. Cytokines &amp; chemokines, Inflammation, Monocyte recruitment, Protease/antiprotease imbalance</th>
<th>Tissue Effects: E.g. GCH, Impaired Mucociliary clearance, Tissue destruction, Collagen deposition</th>
<th>Pulmonary Effects: E.g. Tissue remodeling, Chronic inflammation, Fibrosis, Reduced airflow, Hyperinflation</th>
<th>Lung Disease/COPD: Chronic bronchitis, Emphysema, Small Airways Disease, Increased susceptibility to infection and air pollutants</th>
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</thead>
<tbody>
<tr>
<td><strong>In silico</strong></td>
<td><strong>In vitro (2D): Reporter lines Primary cells</strong></td>
<td><strong>In vitro (3D): Reconstructed human tissues (organoids, spheroids, etc.)</strong></td>
<td><strong>Ex vivo (3D): Whole lungs, precision-cut lung slices</strong></td>
<td>Increasing cost and/or complexity</td>
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Multiple Exposure sites for E-cig extracts, vapors

• Occupational or end user material handling
  – E-liquids contacting skin, eyes?
  • Human 3D Skin Models:
    – EpiDerm ™, Episkin ™, RHE ™ (reconstructed human epidermis)
    – EpiOcular™, HCE ™ (human corneal epithelium)

Regulatory Tox Success Stories:
• Extensive International Testing
• Currently applied toward non-tobacco products
Respiratory Tract Exposures: Vaping

Upper respiratory tract
- Nasal cavity
- Pharynx
- Larynx

Lower respiratory tract
- Trachea
- Primary bronchi
- Lungs

Brown CC10(+) = Club cells

Epithelix’s SmallAir™
MatTek’s EpiAlveolar™

https://en.wikipedia.org/wiki/Respiratory_tract
https://pbs.twimg.com/media/BoA-xHmCcAA50DQ.png:large
https://www.mattek.com/products/epioral-epigingival/
https://www.mattek.com/products/epiairway/
http://www.epithelix.com/products/mucilair
http://www.epithelix.com/products/smallair

EpiOral™
EpiGingival™
EpiAirway™
MucilAir™
E.g. Reconstructed Human Airways

Human reconstructed 3D models (e.g. EpiAirway™ or MucilAir™)

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

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

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

Cells:
- Ciliated
- Goblet
- Basal

Tissue insert at air liquid interface (ALI)

Importance of utilizing compartments (E.g. response after apical exposure)

**IL-6**

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>- Control</th>
<th>Poly I:C</th>
<th>LPS</th>
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<tbody>
<tr>
<td>Apical Rinse</td>
<td>76</td>
<td>100</td>
<td>206</td>
<td>215</td>
</tr>
<tr>
<td>Lysate</td>
<td>228</td>
<td>100</td>
<td>279</td>
<td>39</td>
</tr>
<tr>
<td>Medium</td>
<td>1,792</td>
<td></td>
<td></td>
<td>121</td>
</tr>
</tbody>
</table>

E.g. PCLS: Isolation and Culture

- **Precision-cut lung slice (PCLS) creation is largely conserved**

- **Culture conditions are not!**
  - Nutrient exposure method
    - Rolling vs submerged
    - ALI vs rocking)
  - Culture medium
    - E-199 (IIIVS, USA)
    - DMEM/F12 (Fraunhofer, Germany)
    - MEM (Research Centre Borstel, Borstel, Germany)
    - RPMI-1640 (Bio S&T, Canada)

- **Considering Standardization...**
  - What would a regulator expect?
  - Same results ok, but what if different??
In Vitro/Ex vivo Models: PCLS

PCLS can represent all lung regions present in tissue source.
Aminoflavone Damage

- Exposure of human PCLS to 10 µM Aminoflavone causes cytokine increases in < 24 hr

- Days later, severe tissue damage was noted: AF-induced, decreased cellularity and nuclear changes reflecting toxicity
PCLS: Long Term Culture

- High degree of alveolar and bronchiolar viability retained over 28D
- Some loss of cellularity
• **Inhaled irritants** can activate epithelial cells and **macrophages** to release multiple inflammatory cytokines.

• Prolonged/chronic **inflammation** can result in downstream effects such as **fibrosis** in the small airways.
PCLS: Collagen Deposition (exploratory)

Masson’s Trichrome Stain

- Extensive collagen deposition present in the interior of the PCLS (green arrow).
- Slice margins also show deposition (red arrow).

Control D8

10 mU/ml bleomycin D8

Control D28

100 µM BCNU D28

Large areas of parenchyma exhibit extensive deposition of collagen fibers, especially at slice margins.
Exposure Methods for ALI: solubles

What *Can* and/or *Should* we expose *in vitro* systems to??

TPM, e-TPM, HPHC

**Apical Volume Delivery**

- Repeat exposures?
- Hypoxia?
- Relevance?

**Novel: Digital Dispensing**

- Hybrid technology?
- DMSO or aqueous-based solutions
- Minimal impact on apical rheology
Exposure Methods for ALI

Smoke/Aerosol

- Most relevant!
- Dosimetry?
- Cost!

  e.g.
  PRIT-Expocube
  Vitrocell
  Cultex

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

E-cigarette Research is an Opportunity

- Conducting assays in a calculated, standardized way removes an important variable…how the work was done. **Standardize the assays!**

- Now its time to compare data and accomplish the true objective: **evaluation of biological response** to e-cigarette liquid and aerosol/vapor exposure

- The seemingly infinite combinations of e-cigarette constituents cannot be effectively screened using **in vivo** approaches

- **In vitro** systems can be part of a tiered, cost effective, and highly informative approach

- New Tobacco products are an opportunity to show what may constitute a useful, cost-efficient **Standardized System** to evaluate potential harm
Acknowledgements

- National Academy of Medicine
- IIVS’s staff, supporters & collaborators!
  (standardization & validation doesn’t happen on its own!)

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