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
   2. Focus on 3D

3. E-cigarette Exposures & Examples of Models
   1. Occupational, inadvertent
   2. Vaping

4. Pragmatic use of in vitro/ex vivo Models
   1. Reconstructed human airway tissue
   2. Precision-cut lung slices (PCLS)

5. Exposure Systems

IIVS (non-profit): Three-Pronged Approach

Practical Knowledge (Science)

Dissemination of Information (Education)
Advocacy for the Methods (Outreach)

Increased Use and Regulatory Acceptance

IIVS participated in:
ECVAM-sponsored validation studies, ICCVAM validations, method evaluations, & OECD expert groups to develop in vitro Test Guidelines
Education = Workshops

   - 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

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
In Vitro Models...Scope and Applications

- **Cell lines:** immortalized/transformed cells (BEAS-2B, A549, H292)
- **Primary cells** (normal human bronchial epithelial cells; NHBE)
- **3D cultures/tissues** (organoids, spheroids, reconstructed airway epithelium)
- **Ex vivo tissues** (Precision cut lung slices; PCLS)
- **New technologies** (Lung on a Chip, Human on a Chip, etc.)

**Endpoints:**

- Mutagenicity: Ames assay, micronucleus assay
- Cell stress: Oxidative stress (glutathione levels)
- Cytotoxicity/Viability: Leakage markers, neutral red uptake, MTT
- Inflammation: (ARE, NFκβ, cytokines)
- ...more complex...Mucociliary clearance, tissue changes.
Adverse Respiratory Events & Choice of 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**

**In chemico**

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

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

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

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

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

**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)**

**Increasing cost and/or complexity**
Multiple Exposure Sites for e-Cigarette 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 towards non-tobacco products
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)

- Medium (blood)
  - 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)

Microphotograph source: http://www.epithelix.com/products/mucilair

Importance of utilizing compartments
(Eg. response after apical exposure)

IL-6

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Microphotograph source: http://www.epithelix.com/products/mucilair
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 (IIVS, 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.
Human PCLS: Acute toxicity

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)

Extensive collagen deposition present in the interior of the PCLS (green arrow)

Slice margins also show deposition (red arrow)

Large areas of parenchyma exhibit extensive deposition of collagen fibers, especially at slice margins

Masson’s Trichrome Stain
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

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