In-vitro/ex-vivo exposure system dosimetry: successes and challenges

Symposium: In Vitro Test Methods to Model Local Respiratory Effects after Exposure to Pulmonary Toxicants: Not just smoke and mirrors
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Conflict of Interest Disclosure

- Employee of Altria Client Services LLC
- Altria Client Services LLC has purchased in vitro ALI equipment from Vitrocell Systems GmbH

Outline

- Goal
- Submerged Cultures
- Air-Liquid Interface – Successes
- Air-Liquid Interface – Challenges
- Air-Liquid Interface - Opportunities

Goal

- Know the internal cell dose that results in the observed in-vitro/ex-vivo response?

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- Know the cell surface dose that results in the observed in-vitro/ex-vivo response?

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- Know the cell exposure concentration that results in the in-vitro/ex-vivo observed response?

↑

- Know the intended exposure concentration that results in the in-vitro/ex-vivo observed response?

Submerged Cultures - Successes

- ISDD: (In-vitro Sedimentation, Diffusion Dosimetry) model by Hinderliter et al., 2010 & ISD[3] Thomas et al., 2018

R = gas constant

T = temperature

Na = Avogadro's #

µ = media viscosity

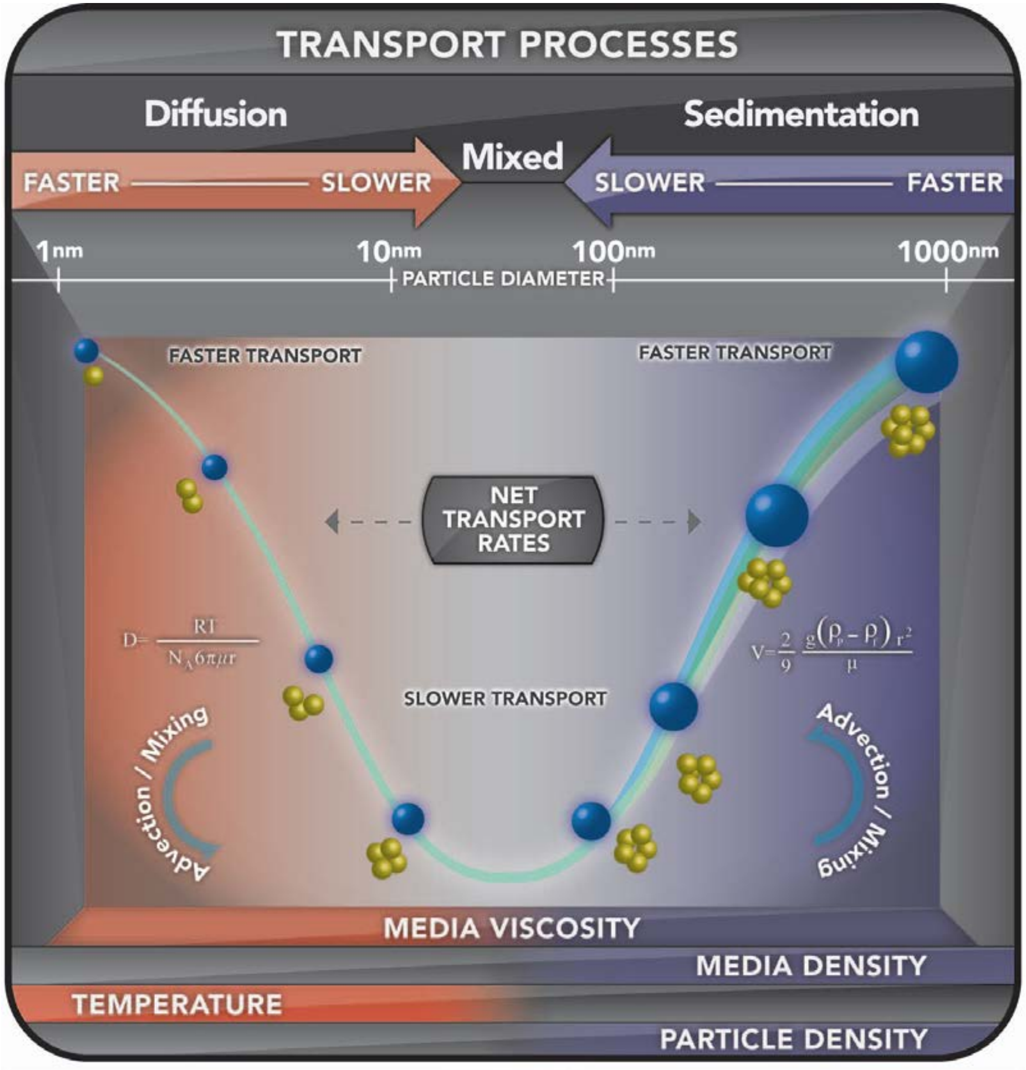
r = particle radius

g = gravitational acceleration

ρ_p = particle density

ρ_f = fluid density

L = total media height



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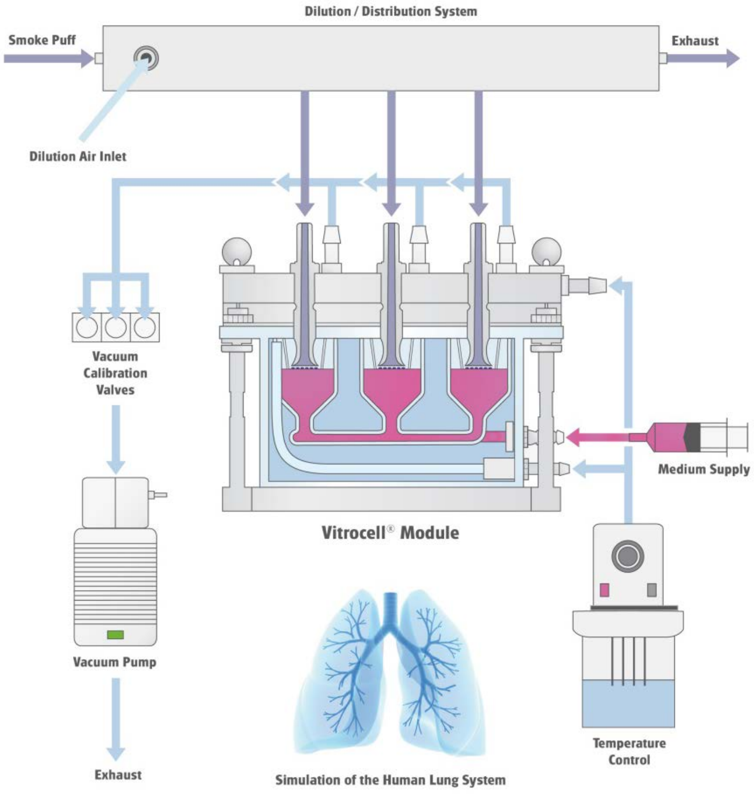
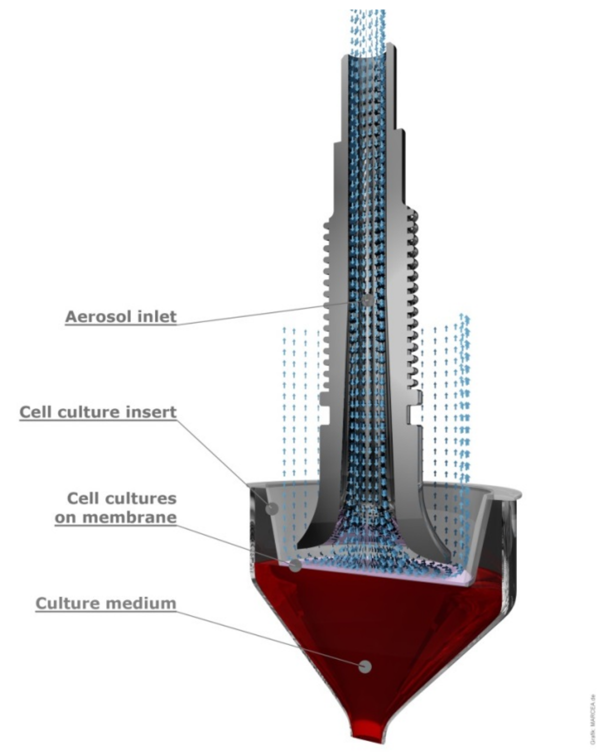
Air-Liquid-Interface

- Diagram of Air-Liquid-Interface exposure system and enlarged diagram of individual exposure cell





Diagrams Courtesy of Vitrocell Systems GmbH

Air-Liquid-Interface - Successes

- Measurement of Particle Deposition
  - Know the cell surface dose that results in the observed in-vitro/ex-vivo response?
    - For a range of solid particles 90 & 196 nm diameter solid particles – TEM using a single cell (Tippe et al., 2002)
    - 7 non-spherical solid particles 29 - 1600 nm diameter particles and 0.1 & 2 µm spherical solid particles - TEM (Comouth et al., 2013).
    - 8 spherical solid particles from 30 – 1005 nm in diameter – TEM in Vitrocell® six cell unit (Fujitani et al., 2015).
    - 5 solid particles from 0.5 – 1.6 µm in diameter – fluorescence - Vitrocell® 24/48 system (Steiner et al., 2017a,b).
    - 42nm & 40µm – gravimetrically (Aufderheide et al., 2017) in a Cultex® radial flow unit.
    - mainstream tobacco smoke - spectrofluorometric quantification in a custom exposure system (Adamson et al., 2011)

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Air-Liquid-Interface - Successes

- Measurement of Particle Deposition
  - Know the cell surface dose that results in the observed in-vitro/ex-vivo response?
    - Quartz crystal microbalances (QCM) - Successes
      - Changes in vibration frequency are correlated to amount of mass on the crystal (Sauerbrey, 1959) with 3 assumptions:
        - Mass deposited is small compared to the mass of the quartz crystal
        - Mass is rigid
        - Mass is evenly distributed over the crystal surface
      - Excellent sensitivity, depends on crystal thickness ~10 ng/cm^2
    - Mainstream tobacco smoke (Adamson et al., 2012, 2013 a,b; Thorne et al., 2013; Majeed et al., 2014;)

Air-Liquid-Interface - Successes

- Measurement of Particle Deposition
  - Know the intended exposure concentration that results in the in-vitro/ex-vivo observed response?
    - Photometers
      - Light scattering is converted to aerosol mass or particle size distribution
      - Refractive index of aerosol will affect readings
      - TPM from mainstream tobacco smoke (Ritter et al., 2003)

- Know the cell exposure concentration that results in the in-vitro/ex-vivo observed response?
  - Chemical Identification
    - Vitrocell® 24/48 - mainstream tobacco smoke (Majeed et al., 2014)
      - Nicotine and metabolites from PBS below cells
      - Selected carbonyls from line exhaust
    - Cultex RFS module – mainstream tobacco smoke (Ishikawa et al., 2016)
      - Solanesol - modified insert with trapping solution at cell level
      - Acetaldehyde – modified insert with trapping solution at cell level

Air-Liquid-Interface - Successes

- Know the cell exposure concentration that results in the in-vitro/ex-vivo observed response?
  - Chemical Identification
    - 6 cell exposure module (manufacturer not specified) – mainstream tobacco smoke (Fields et al., 2017)
      - TPM – trapping solution at cell level
      - CO – online monitor

Air-Liquid-Interface - Challenges

- Liquid droplet aerosols (e-vapor product aerosols)
  - QCM not suited for measurement
  - Photometers - depending on exposure flowrate, aerosol concentration exceeds capacity
  - Significant amount of semi-volatile constituents (Ingebrethsen et al., 2012)
  - Differences in constituent refractive and constituent volatility could affect readings

- Relative Humidity > 75-80% RH (Zavala et al., 2017)
  - Most publications don't indicate RH
  - If they do, the RH existing the humidification system not exiting the exposure system is usually stated

Air-Liquid-Interface - Challenges

- Temperature = 37ºC (Zavala et al., 2017)
  - Most publications indicate that cells are kept at 37ºC but don't indicate the temperature of the exposure atmosphere
  - Difficult to raise temperature of exposure air due to small residence time in exposure system

- Thermophoretic Effect
  - Temperature difference betweem exposure air and cells creates a thermal barrier to deposition (Neilson et al., 2015; Higuchi et al., 2016)
  - The lower the exposure flowrates and the greater temperature difference the larger the thermal barrier

Air-Liquid-Interface - Challenges

- Equipment Operation
  - Preliminary studies yield unexpected results in a Vitrocell 24/48 system for deposition of 3.2µm monodisperse fluorescent particles
    - Between 8,000 – 10,000 particles found on filters at the end of each row
    - Media/phosphate buffered saline below inserts was not used

Transwell ID	Particle Counts	Transwell ID	Particle Counts	Transwell ID	Particle Counts	Transwell ID	Particle Counts
A1	132	A2	2	A3	166	A4	4
B1	162	B2	0	B3	144	B4	0
C1	170	C2	0	C3	139	C4	54
D1	53	D2	90	D3	1	D4	151
E1	1	E2	102	E3	0	E4	149
F1	4	F2	113	F3	0	F4	149

Air-Liquid-Interface - Challenges

- Equipment Operation
  - Lagrangian approach using Fluent® CFD software for air velocity profiles – added fluid channels below transwells to simulation

VC24_Channel_D2_2cc.mp4

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Air-Liquid-Interface - Opportunities

- Quartz crystal microbalance with dissipation (QCM-D) - Challenges
  - Provides novel insight into viscoelastic properties of deposited mass (liquids and vapor: Vashist & Vashist, 2011)
  - With specific coatings has been used to measure specific volatile organic chemicals, ammonia, benzene, etc. (Vashist & Vashist, 2011)

- New Photometers
  - Dual lasers with polarized light designed for e-vapor products (Wang et al., 2017)

- Thermophoretic Effect
  - Use thermophoresis to drive deposition (heat the exposure air)
  - How realistic and how will cells respond?

Air-Liquid-Interface - Opportunities

- Electrostatic precipitation
  - Used for decades to collect ambient airborne particulate matter
  - For Bipolar charged 50 – 600nm particulate, 15-35% enhancement of particle deposition is possible (Savi et al. 2008)
  - Gaschen et al., 2010 and de Bruijne et al., 2009 have reported on custom made systems
    - Up to 90% deposition for 19-882nm particles (de Bruijne et al., 2009)
    - No cellular effects noted due to the electric field (de Bruijne et al., 2009)

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