

In Vitro Testing of Aerosolized Substances with Primary 3D Human Airway Epithelia: Application of ALICE-Cloud Technology to e-liquids (e-cigarettes)

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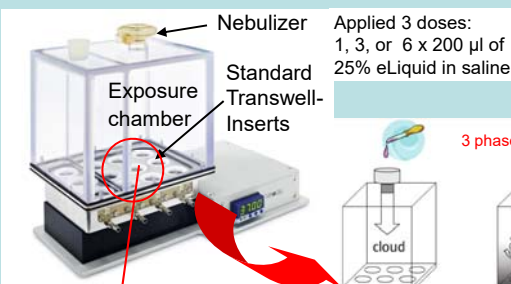
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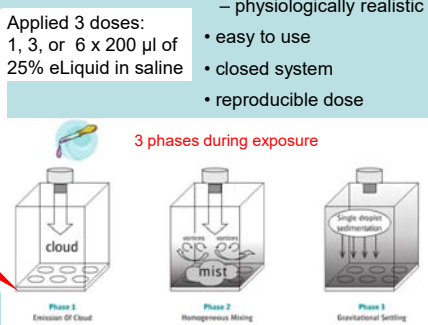
Methodology for Aerosolized Application of eLiquids to Cells Cultured at the Air-Liquid Interface (ALI)

Aerosol-Cell Exposure System (VITROCELL-Cloud (VITROCELL, Germany))



Advantages

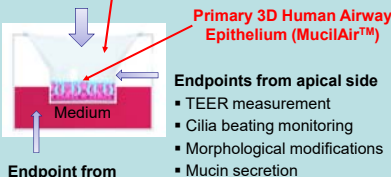
- cultured at air-liquid interface (ALI) – physiologically realistic exposure
- easy to use
- closed system
- reproducible dose



Lenz et al., 2014)

Cells & Biological Endpoints (MucilAir™, Epithelix, Switzerland)

Aerosol (1.5, 4.5 or 9 µl per 12-well insert)



- Primary 3D Human Airway Epithelium (MucilAir™)
- Endpoints from apical side
- TEER measurement
 - Cilia beating monitoring
 - Morphological modifications
 - Mucin secretion

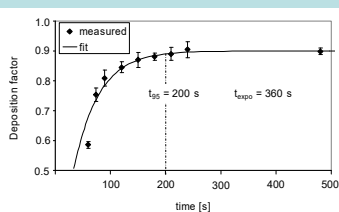
Endpoint from basolateral side

- IL-8 secretion

24h post-exposure incubation

Aerosol Delivery: Time Course

Aerosol Delivery Versus Time



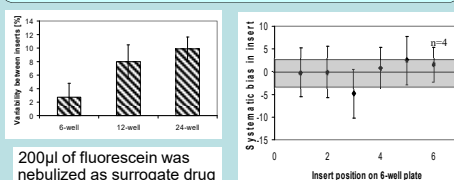
- 25% eLiquid in saline: 0.2 ml nebulized
- Total exposure time: $t_{95} = 200$ s (typically used 360 s)
- Liquid film thickness on cells: ~13 µm for 1x 0.2 ml of aerosolized liquid (39 µm or 78 µm for 3 or 6 exposures)

References

- Lenz, A.G., et al., Efficient bioactive delivery of aerosolized drugs to human pulmonary epithelial cells*Am. J. Resp. Cell Mol. Biol.*, 51, 526–535, 2014
- Constant, S; et al., The use of in vitro 3D cell models of human airway epithelia (MucilAir™). *Cellular In Vitro Testing - Methods and Protocols Chap. 2*, 2014

Aerosol Delivery: Dose/Variability

Uniformity of Aerosol Distribution Amongst Inserts

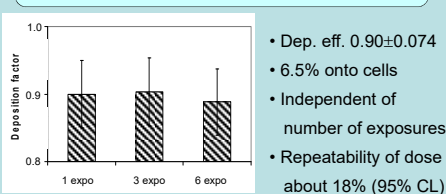


200µl of fluorescein was nebulized as surrogate drug

Well-to-well variability of drug dose:

- Between 6 and 20% (95% CL) depending on type of multiwell plate (left panel)
- No systematic bias in any of the wells: Mean +/- SEM of deposited dose is within expected well-to-well variability (95% CL; right panel)

Substance Efficiency and Repeatability of Delivered Dose

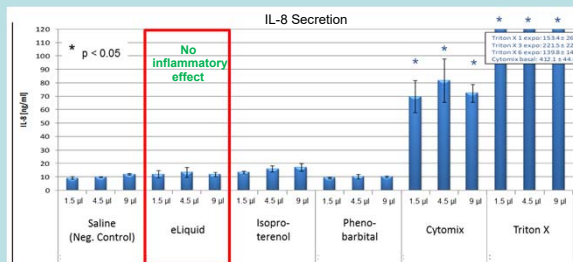
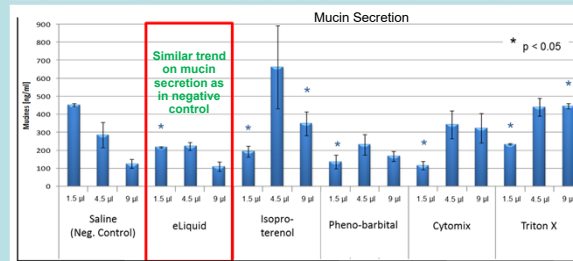
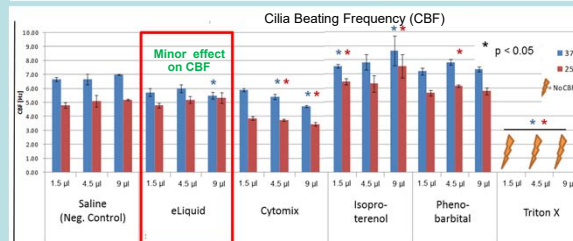
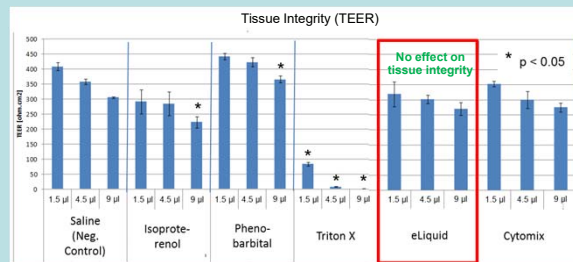


- Dep. eff. 0.90 ± 0.074
- 6.5% onto cells
- Independent of number of exposures
- Repeatability of dose about 18% (95% CL)

Substances: Composition-Response/Summary

Sample	Composition	Results/Summary
E-liquid	75 % Propylene glycol 15 % Vegetable glycerin 10 % H ₂ O diluted 1/4 in Saline Sol.	No significant adverse effect (except: Mucin secretion ↓ (for lowest conc.))
Cytomix	0.4 mg/ml LPS 5 µg/ml TNF-α 1 % FBS in Saline Sol.	Cilia beating frequency ↓ IL-8 secretion ↑
Isoproterenol (cilia activator)	2 mM in Saline Sol.	Cilia beating frequency ↑ Mucin secretion ↑
Phenobarbital (cilia inhibitor)	10 µg/ml in Saline Sol.	Mucin secretion ↓ (lowest conc.) IL-8 secretion ↑
Triton X	1 % Triton X 100 in Saline Sol.	No effect on cilia beating! TEER & Cilia beating frequ. ↓ (completely eliminated) Mucin secretion ↑ IL-8 secretion ↑

Results



Conclusions/Summary

- ALICE-Cloud technology: Easy to use, **commercially available system** (Vitrocell Systems, Germany) for aerosolized delivery of liquids to cells cultured at the air-liquid interface (ALI)
- Combined with primary ALI 3D cell cultures - Physiologically more realistic lung model than submerged cell systems
- Closed system, small enough to be operated under clean bench conditions
- Efficient drug deposition on bottom plate of ALICE-CLOUD: 0.90 ± 0.074
- Due to limited cell coverage in multi-well plate: ~6.5% cell-delivered dose
- Cell delivered dose rate: 1.5 µl per insert per exposure
- Time for performing one complete cell exposure: ~ 5-7 min (200µl liquid)
- Even **very high eLiquids doses** (~ smoking of 500, 1500 and 6000 e-cigarettes within 7 min) had almost **no adverse effects** on human primary bronchial epithelial cells in terms of tissue integrity, cilia beating frequency, mucin secretion and IL-8 secretion.