NTP
National Toxicology Program

Overview of *In Vitro* Exposure Systems

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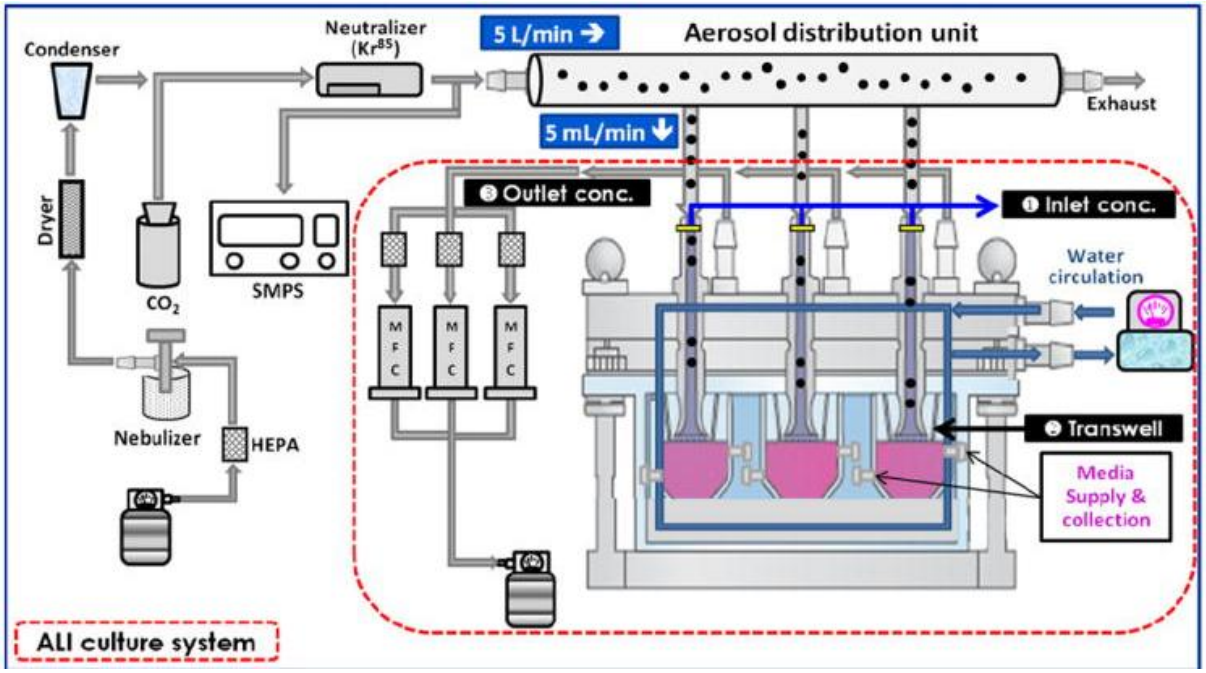
Integrated Laboratory Systems / NICEATM

General

- Generator: creates an aerosol
- Connections and peripherals: tubing, conditioning devices and measurement devices
- Exposure Chamber: Houses cells



Generator – Charge Questions

1. Which aerosol generation technology is suitable for nanomaterials (NMs), especially MWCNTs/fibres? Which methods are incompatible with MWCNTs/fibres? Can data (published or experimental) be provided to demonstrate the superiority of the method?
2. How long is the aerosol stable?
3. What relevant benefits and limitations does each system have (see table in dropbox for full list)?

- Compatibility with other NM types
- Compatibility with non- NMs
- Ease of cleaning / Sterility

Generator - Technology

Dry

- Fluidized Bed
- Acoustic
- Feeders: brush, dust and turntable

Wet

- Nebulizer
- Electrospray

Generator - Technology

Dry

- Moisture adjustment required
- More Heterogeneous Size
- Modest [ ] range
- Aerosol of “regulatory concern”

Wet

- Need to identify compatible solvent
  - CNT deagglomeration issues
- Uniformity in Size
- Very High [ ] possible

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Exposure - Goal

- Replace a Complex system:
  - A chamber
  - A monitoring system
  - An animal
    - Tissue of Deposition
    - Tissue of Disease
    - Humidity control
    - Temperature control
    - Dosimetry control (size and amount)

Exposure – Charge Questions:

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3. What method will be used to analyze the cellular dose?

4. Which of the presented benefits and limitations are relevant?

- # of samples: Will it require multiple systems?
- Modularity: Is it compatible with other NMs and non-NMs?
- Ease of cleaning / Sterility

Exposures – System Features

Membrane material	variable (polystyrene)	Compatible with transwell inserts, so membrane material can be selected	Compatible with transwell inserts, so membrane material can be selected	Compatible with inserts from different suppliers (Corning or Millipore, which are also used by MatTek) or petridishes	Commercial 12-well multiwell plate with ALI cultures on membranes of the Corning or Becton Dickinson types (growth surface area 1cm²). Applicability to cell lines or primary cultures, complex 3D cell cultures models or ex vivo tissue (precision cut lung slices, PCLS)
Can cells be grown on a transwell filter or equivalent, with separate media above and below?	yes	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system
Well size	variable (well plate, petri dish, chamber)	6 or 12 well plate	24 well	variable (inserts or petridishes)	
Can cells be grown at an air liquid interface?	yes	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system	yes. Because transwells are compatible with the system
Can cells be exposed to an aerosol?	yes	yes	yes	yes	yes
Length of culture	up to 7 days with L2, A549, BEAS2B, U937, JAWSII	up to 24 h reported	up to 24 h reported	up to 24 h reported	
Has the system ever been used for lung epithelial culture?	yes	yes. A549 cells (Frohlich et al, 2013; Kim et al, 2013), MucilAir, A549 or BEAS-2B cells (Kooter et al)	yes. BEAS-2B (Jeannet et al, 2014)	yes. A549 cells (Aufderheide et al, 2013; Steinritz et al, 2013)	yes
Has the system been used for other epithelial cells?	yes				
Can the system be used to measure trans-epithelial electrical resistance TEER?	yes	The transwells will have to be moved to plates to meaure TEER under submerged conditions	The transwells will have to be moved to plates to meaure TEER under submerged conditions	The transwells will have to be moved to plates to meaure TEER under submerged conditions	
Can the size of the system be altered (ie can the size of the culture be changed)?	Can be modified to fit any sized well plate (or petri dish)	yes			
Has the system ever been used with nanomaterials? Which NMs? (material type)	yes	yes. Polystyrene particles, carbon nanotubes (Frohlich et al, 2013), Cerium dioxide (Kooter et al.), Copper (Kim et al, 2013)	yes. Silver, polystyrene (Jeannet et al, 2014), carbon-based (Kunzi et al, 2013), Adipic acid nanoparticles (Mertes et al, 2013)	yes. Copper oxide (Aufderheide et al, 2013, Steinritz et al, 2013)	yes (particles between 389nm and 2019nm have been tested in the system to date).
Mode of nanomaterial deposition (electrostatic or gravitational)	Compaction	Electrostatic/gravitational	Electrostatic	Electrostatic or gravitational. For electrostatic we will need Cultex Electrical Deposition Device	
Deposited-dose determination		One of the systems (not the cloud system) has an option to measure the weight of the particles	At least one of the wells is designed to carry a TEM grid to monitor exposure	The deposition of the particles can be analyzed by gravimetric methods, using the precision balance (SE2-F filter ultra-microbalance).	
Is the system suitable for radioactive particles?	Unknown	Unknown	Unknown	Unknown	Unknown
Is the system reusable or disposable?	reusable	reusable	reusable	reusable	reusable
What is the cost of the system?	$25,000	See "VitroCell Quote" tab	See "NACIVT Quote" tab	See "Cultex Quote" tab	
What level of throughput (either number of samples or doses) and content screening (level of multiplexing) can be achieved?	Expose one chamber at a time; high content; variable dose; multiple cell types	Expose six chambers at a time; high content based on the number of units used	24 wells	Has three exposure chambers	8 wells based on information available on manufacturers website
What level of cellular complexity can be achieved (i.e. multi-cell cultures which mimic alveolar or airway)	Complex	Complex	Complex	Complex	Complex
Commercially available	no	yes	no (U Bern makes 5 units at a time and they start making them when they have 3 orders submitted.	yes	yes
Target aerosol size distribution	Tunable				
Exposure time duration	Tested up to 3 hours	I hour exposure (as tested in Fröhlich et al., 2013), Maximum 4 h possible (as tested in Kim et al., 2013, exposure beyond 4 h lead to decrease in cell viability)	upto 2 h reported (Mertes et al., 2013; Jeannet et al., 2014)	Exposure experiments with 4 hours have been done (per manufacturer). The maximum length depends on the flow of the atmosphere over the cells and on the test atmosphere. Upto 1h (Aufderheide et al., 2013, Steinritz et al., 2013)	
Method for maintaining humidity	yes	Yes. Computer controlled (aerosol can be humidified)	Yes. Computer controlled	The humidity depends on the test atmosphere but basically there is a humidity of >90% directly over the cells due to evaporation.	
Temperature maintained	yes	yes	Yes. Computer controlled	CULTEX® RFS is heated by an external water bath, the CULTEX® RFS Compact has an integrated heating device with temperature control	
Multiple chambers for multiple exposures	yes	yes	yes. 24 wells can be exposed at the same time	yes. The standard CULTEX® RFS has only three test chambers (6.5, 12 and 24mm cell culture inserts and Petri dishes) and the smaller CULTEX® RFS Compact has 6 chambers (6.5 and 12mm cell culture inserts)	yes
Capable of both liquid droplets and solid particles	yes	yes	yes	Yes. based on brochure.	
Ability to utilize multiple aerosol generating techniques	yes	yes	Can connect NACIVT to any aerosol. Aerosols can be generated by spark generation, nebulization etc. Can be placed close to the workplace or aside a busy street.		
Uniform particle deposition across the transwell insert	yes, still working on achieving uniform deposition of NMs	yes, according to manufacturer	yes, according to manufacturer	yes, according to manufacturer	
High collection efficiency					
Aerosolization of native material without solvent	yes	yes	yes because fo the flexibility in using different types of the aerosol generators	Yes. Cultex DG is used to aerosolize nanomaterials.	

Exposures – System Feature Needs

Physics of Delivery

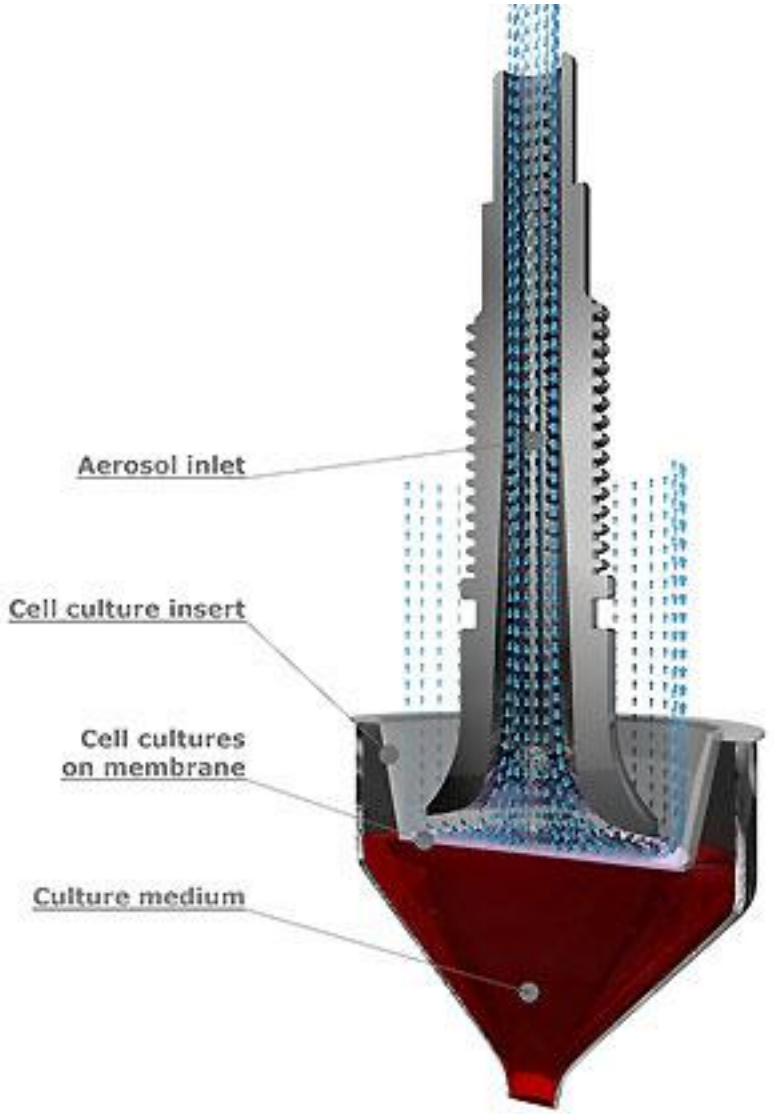
- Deposition of Nano-scale aerosol
- Accurate/Precise/Reproducible Delivery
  - To the chamber
  - To the cells
- Sampling Capability

Regulatory Suitability (Design)

- # of replicates / doses needed
- QA/QC compatibility
- Containment of Hazards

Biological Suitability

- Size of insert must generate sufficient sample
- Flexibility
- Maintenance of Cells – long term ALI culture
  - Sterility



In Vitro Exposure Systems

The Equipment

	VitroCell	Cultex	NACIVT
Deposited-dose determination	VitroCell 6 CF and 12 CF have optional microbalance	Gravimetric methods, using the precision balance.	TEM grid to monitor exposure.
What level of throughput can be achieved?	**VitroCell 6 CF** •3, 4 or 6 samples •6 well inserts **VitroCell 12 CF** •3 or 4 •12 well inserts	**RFS** •3 samples •6, 12, or 24-well inserts **RFS Compact** •6 samples •12, or 24-well inserts	•24 samples •6 well inserts
Ability to utilize multiple aerosol generating techniques?	Modular construction allows connection to any generator.	Modular construction allows connection to any generator.	Not modular beyond generator.
Commercially available?	yes	yes	no (5 units at a time).
Exposure time duration	Maximum 4h possible	Up to 1h reported	Up to 2h reported
Pubmed search by name	17	31	1

Exposures – Divergence Points

Non-Distinguishing

- Compatible with CNTs
- Usable with Transwells
  - ALI compatible
- Modular Generator
  - Aerosol can be conditioned

Distinguishing

- Deposition Analytics
- Variable Configuration and Form factor
- Modularity of peripherals
  - Aerosol conditioning: NACIVT

Exposures – Divergence Point 1

Non-Distinguishing

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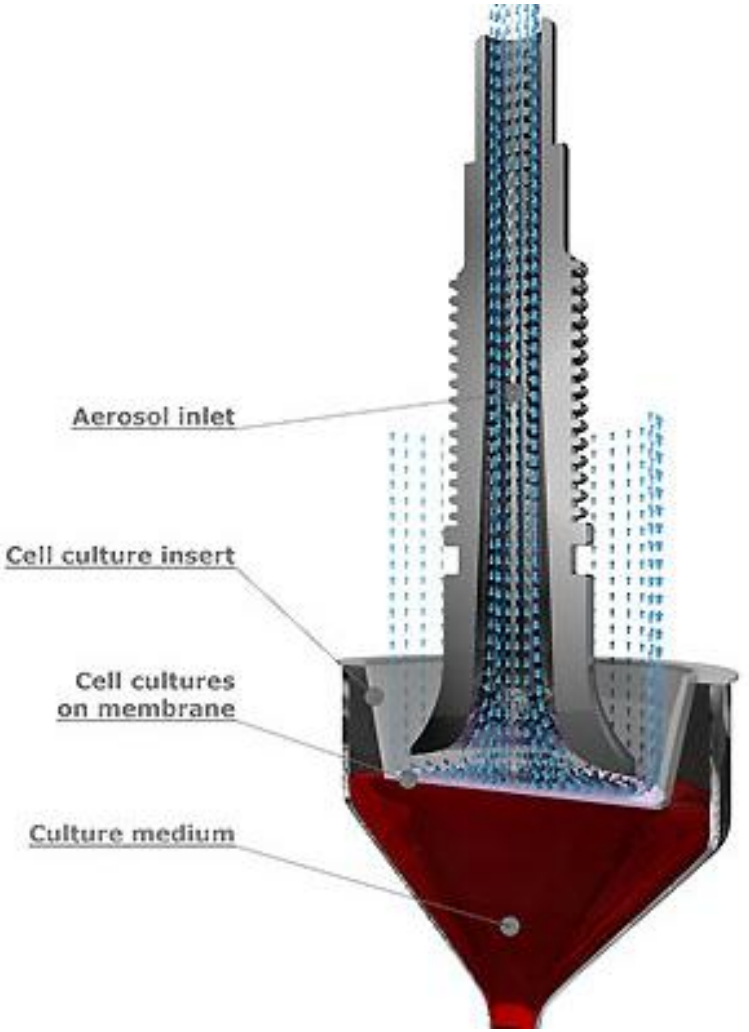
Exposures – Physical Divergence

- Will electrostatic deposition be needed?

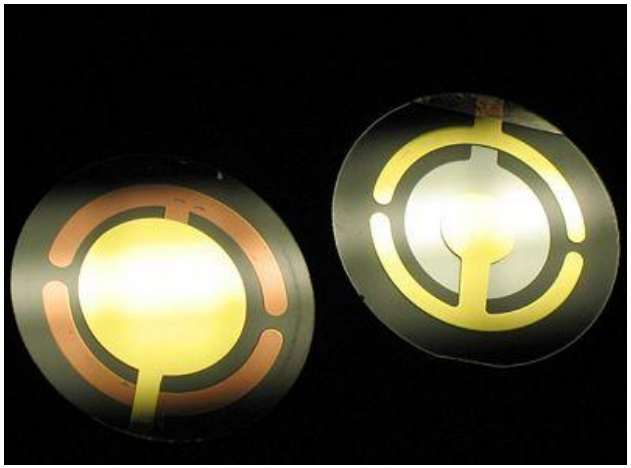


Exposures – Physical Divergence

Mass or Particle Based?

- Mass
  - Rapid Resolution
    - Real Time
    - Needed along with aerosol characterization?
  - Cheap
    - Particle-based still necessary
- Particles:
  - Dose fraction translatability
  - Hazard interpretability
  - Needed with aerosol & cellular characterization?

Which is easier to add later?



Exposures – Physical Divergence

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Exposures – Divergence Point 2

Non-Distinguishing

- Compatible with CNTs
- Usable with Transwells
  - ALI compatible
- Modular Generator
  - Aerosol can be conditioned

Distinguishing

- Deposition Analytics
- Variable Configuration and Form factor
- Modularity of peripherals
  - Aerosol conditioning: NACIVT

Exposures – Biological / Regulatory Divergence

	VitroCell 6 or 12 / x	VitroCell 12/12 or 24	Cultex RFS	Cultex RFS Compact	NACIVT
What size inserts?	6 ; 12 well	24 ; 48 well	6, 12, or 24-well	12 or 24 well	6 well
How many replicates per exposure condition?	1-6*	4 ; 6	3	3 or 6	24
How many 'chambers' on a unit?	3, 4 or 6	6 ; 8	3	6**	24
Media delivery?	Static or Flow	Static	Static or Flow	Static	Static
Temperature Control?	Water Jacketed	Electronic Heating	Water Bath and Pump	Water Bath and Pump	Electronic Heating
Other included functionality	None - Electrostatic deposition only on 6 series	None	None	None	Electrostatic deposition, humidifier, aerosol charger, etc.

Exposures – Design Cost / Benefit

Large Sample volume

- Multi-analysis Capable
- Ample Sample for Analytics

Large # of Conditions or Replicates

- Dose Response
- Multiple Conditions
  - Condition Controls
  - Biological Controls

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Exposures – Divergence Point 3

Non-Distinguishing

- Compatible with CNTs
- Usable with Transwells
  - ALI compatible
- Modular Generator
  - Aerosol can be conditioned

Distinguishing

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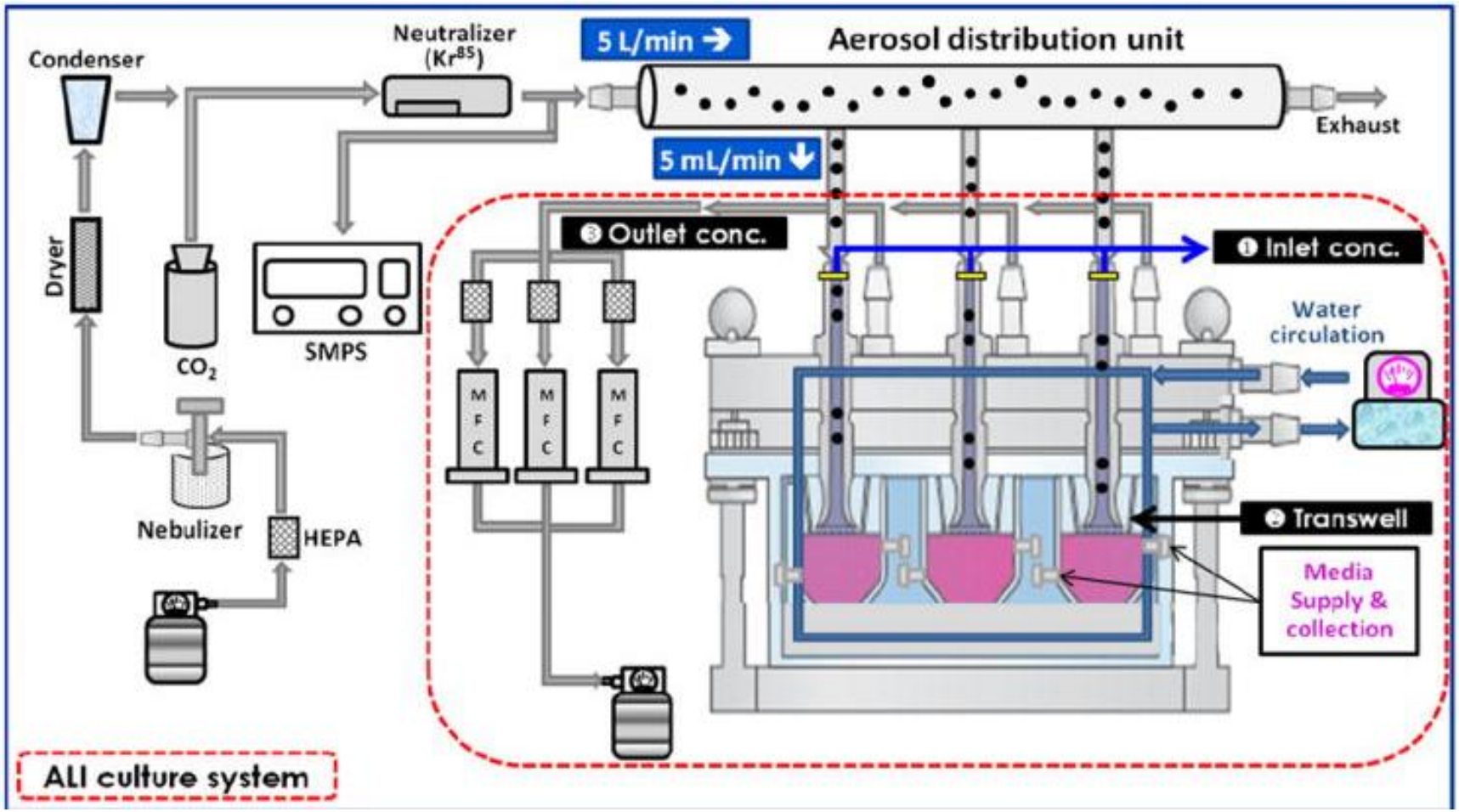
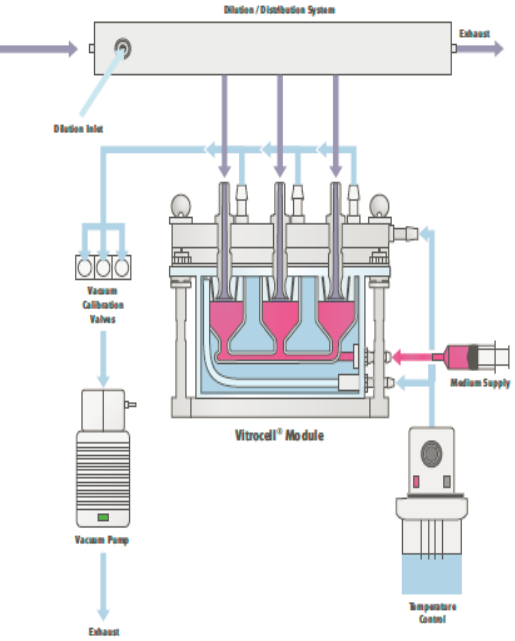
  - Aerosol conditioning: NACIVT

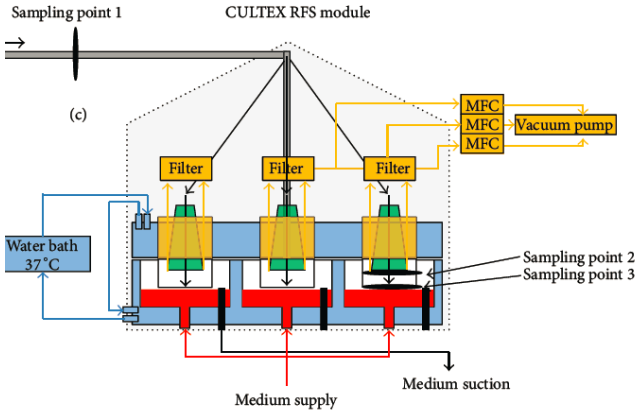
Exposures – Modularity

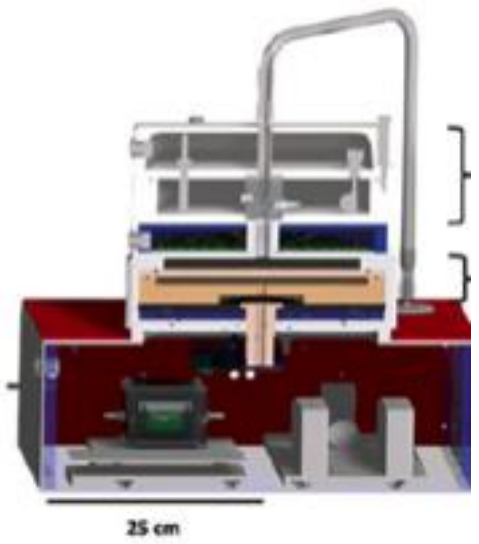


Toxicol In Vitro. Feb 2013; 27(1): 164–173.

Exposures – Modularity







VitroCell 6 & 12 > Vitrocell 24=Cultex >>> NACIVT

Exposures – Modularity

Vitrocell / Cultex: Modular

- Easier to troubleshoot?
- P-chem tunable
- Physiology is tunable

NACIVT: All-in-one

- Easier for deployment?
  - Portable
  - Cost to size ratio
- Already very robust
  - Known limitations?
- Physiologically relevant to deep lung

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Exposures – Limitations in all current systems

- Sterility is Process dependent
- Fluid Dynamics
  - No laminar liquid flow in sub-compartment
  - Alveolar fluid regulation at the ALI?
- Low throughput in all
- Documented long term exposure
  - Limited or no repeated dose examples
  - Reported short exposures only (<4 h)
  - Sterility not a design feature of any system

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