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Vicki Stone

Longer term ideas for developing *in vitro* models for pulmonary toxicology

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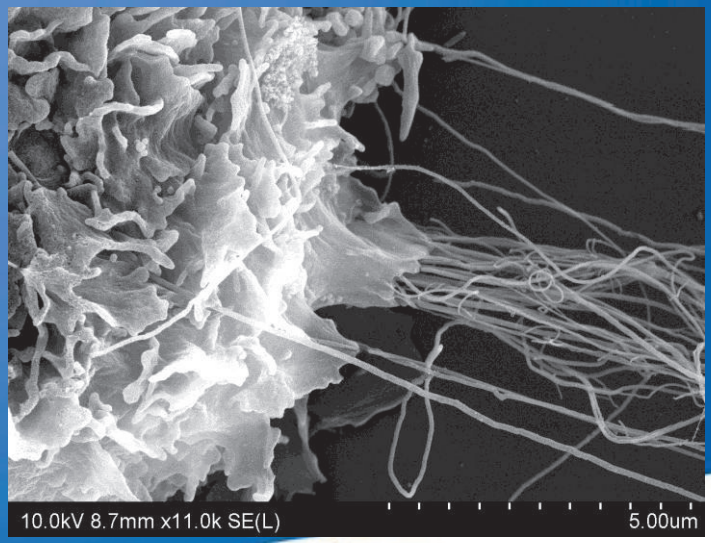

THE SUNDAY TIMES SCOTTISH UNIVERSITY OF THE YEAR 2011-12 HERIOT-WATT UNIVERSITY

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Distinctly Ambitious
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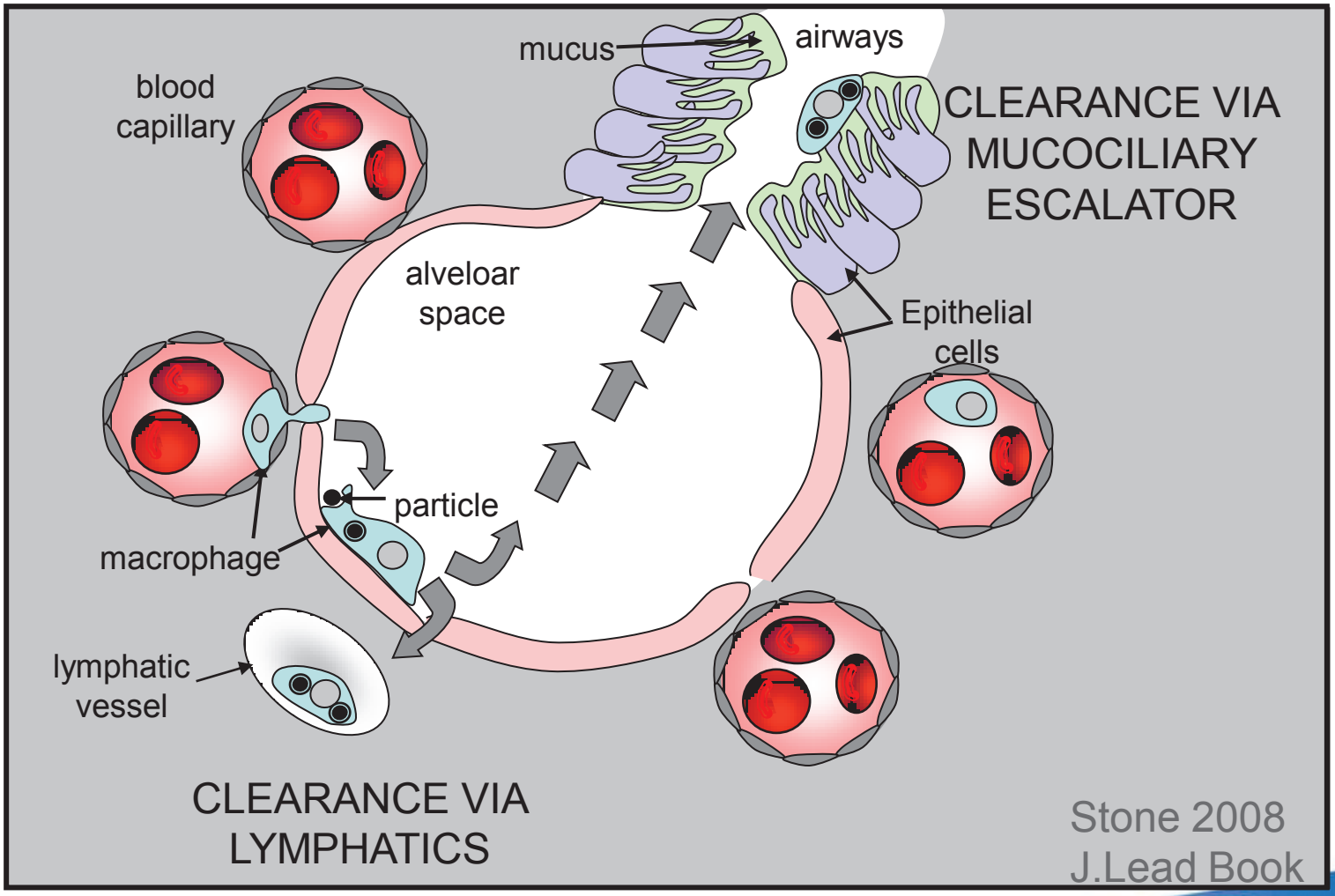
Overview

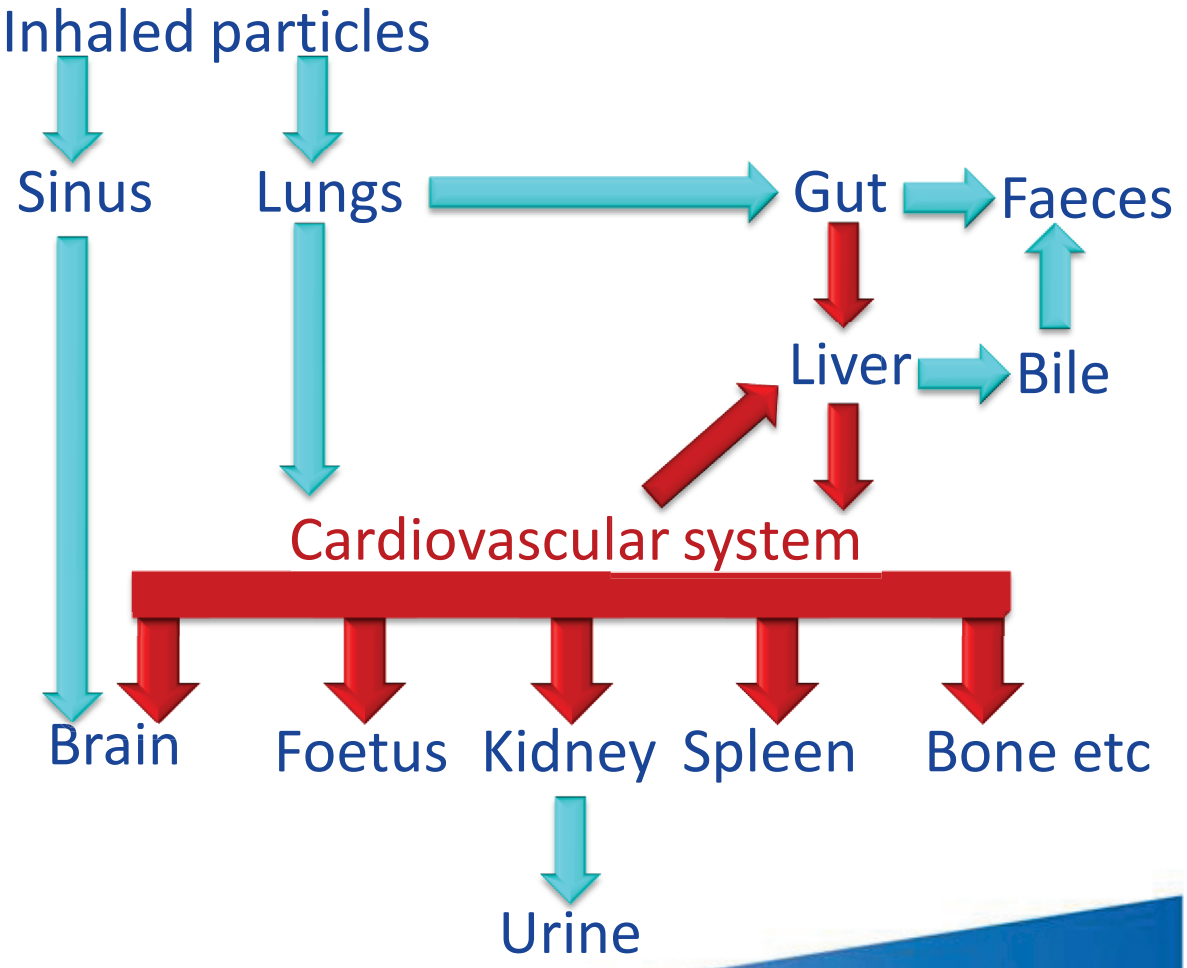
- The workshop has concentrated on an achievable first generation lung model.
  - Multiple cell types (epithelial and inflammatory)
  - Aerosol generation
  - NM's to investigate
  - Endpoints and cell systems

Can we take the suggested *in vitro* models even further?



Stone 2008 J.Lead Book

Particle uptake and impacts on distal organs



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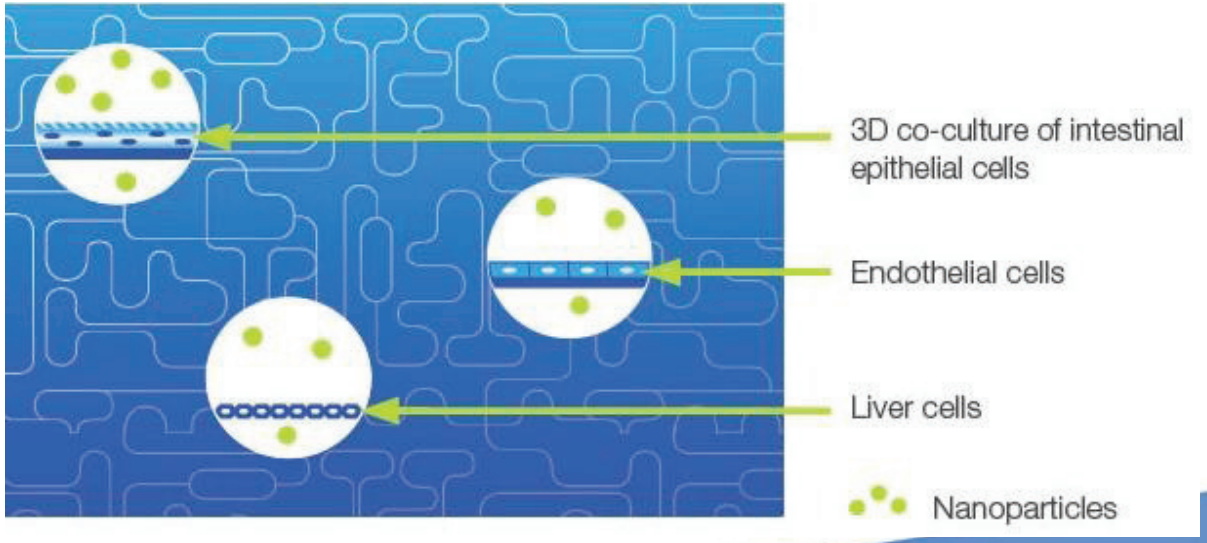
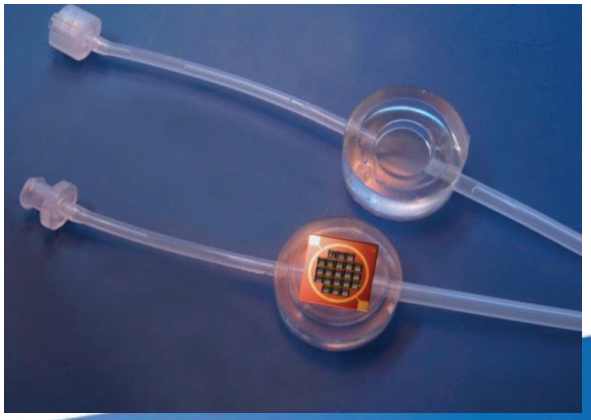
How can we further improve models in the future?

- Microfluidic models and lab on a chip technologies offer the potential to consider
  - More complex 3 dimensional multi-cellular models
  - Interactions between different tissue types
  - Translocation and distal target effects
  - Something that better reflects the physiological, toxicological and pathological reality
  - Development of models to reflect diseased status
  - Primary cells
  - Genetic diversity…

Making the ATS more relevant

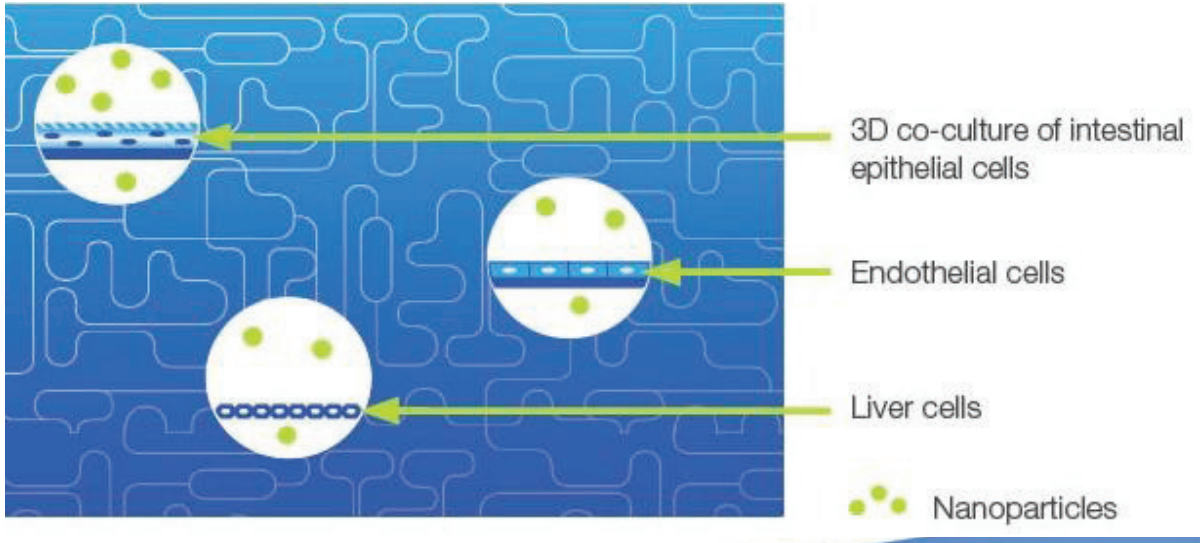
- InLiveTox (EC FP7)
  - Aim to generate a microfluidic system to investigate uptake via ingestion and impacts of NM
    - Differentiated Caco-2 gut epithelium ± macrophages
    - Endothelial cells
    - Hepatocytes

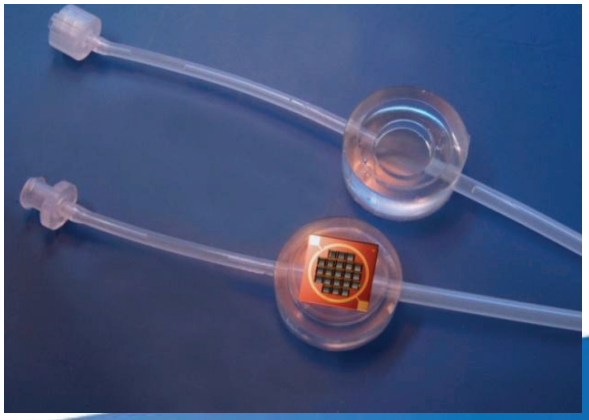




What did InLiveTox achieve?

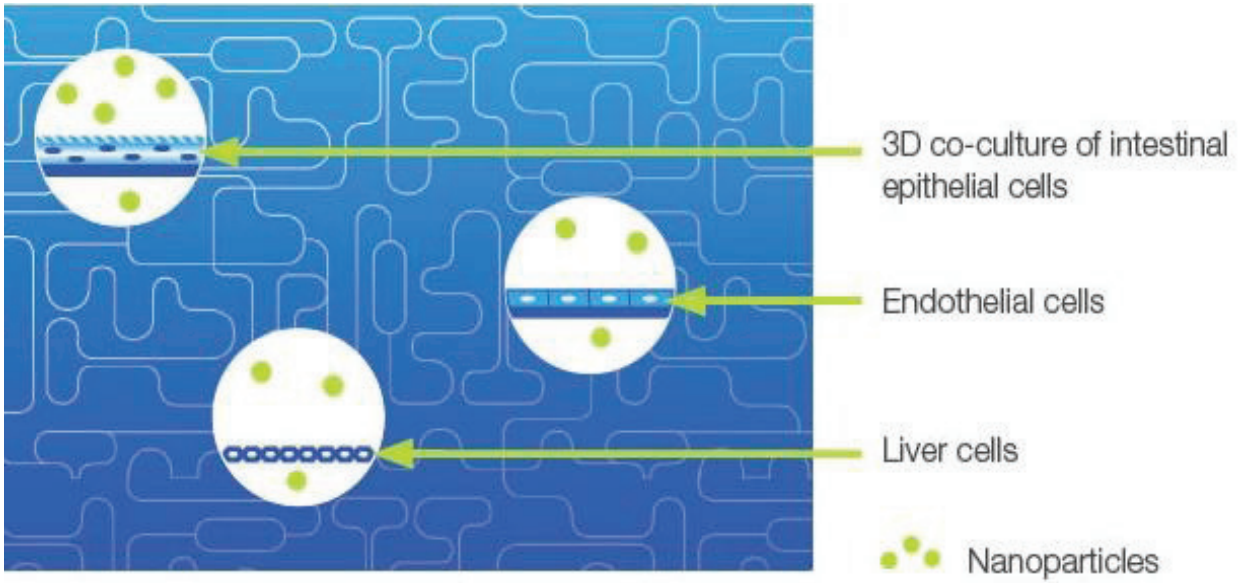
- A common culture medium for gut epithelium, macrophage, endothelial and hepatic cell lines
- Continued viability of each cell type under flow conditions for up to 72 hours
- Apical and basolateral exposure of the gut epithelium to NP
- Assessment of molecular, biochemical and microscopic responses of each cell type to the NP exposure
- A portfolio of protocols





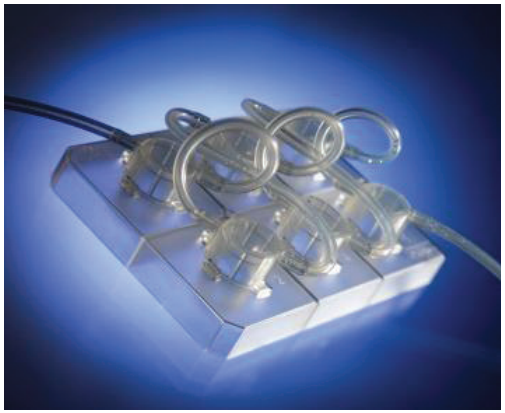
Could InLiveTox be adapted to represent the pulmonary exposure route?

- Switch the Caco2 cell line/macrophage coculture for the system developed from this workshop?



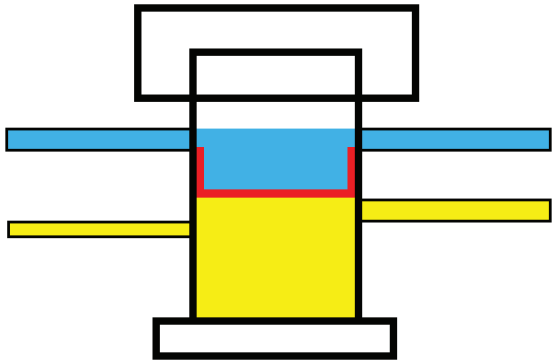
Microfluidic system - Kirkstall

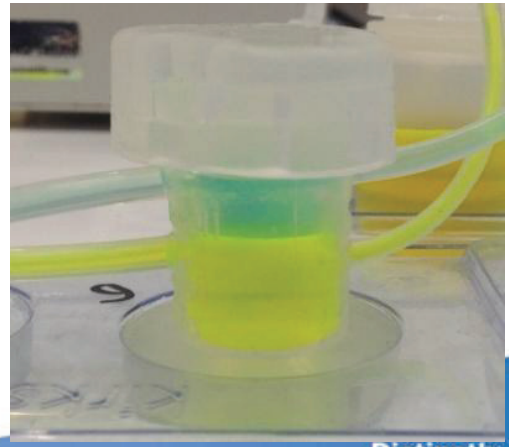
- Quasi-Vivo system
  - Modular
  - Flexible
  - Disposable
  - Sterile



Microfluidic system - Kirkstall

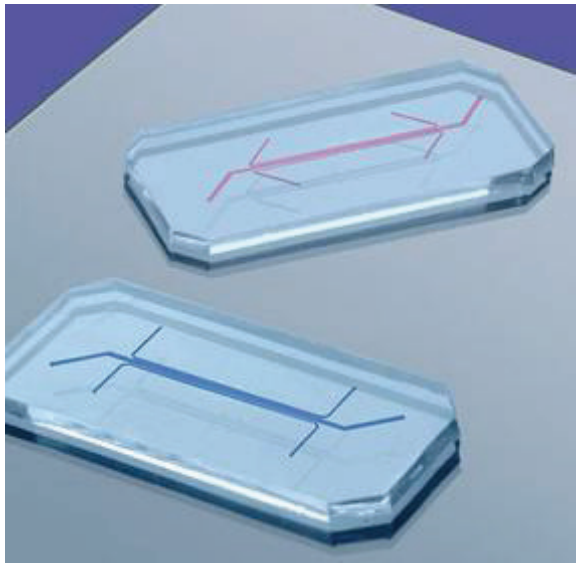
- Cells are grown on an insert
- Fluid flow controlled above and below membrane simultaneously, but separately
- Can sample from apical and basolateral surface any time
- Can make additions to apical and basolateral surface any time
- TEER compatible
- Aerosol exposure – why not?
- Liquid and cell volumes adequate for molecular, biochemical and microscopic analysis.





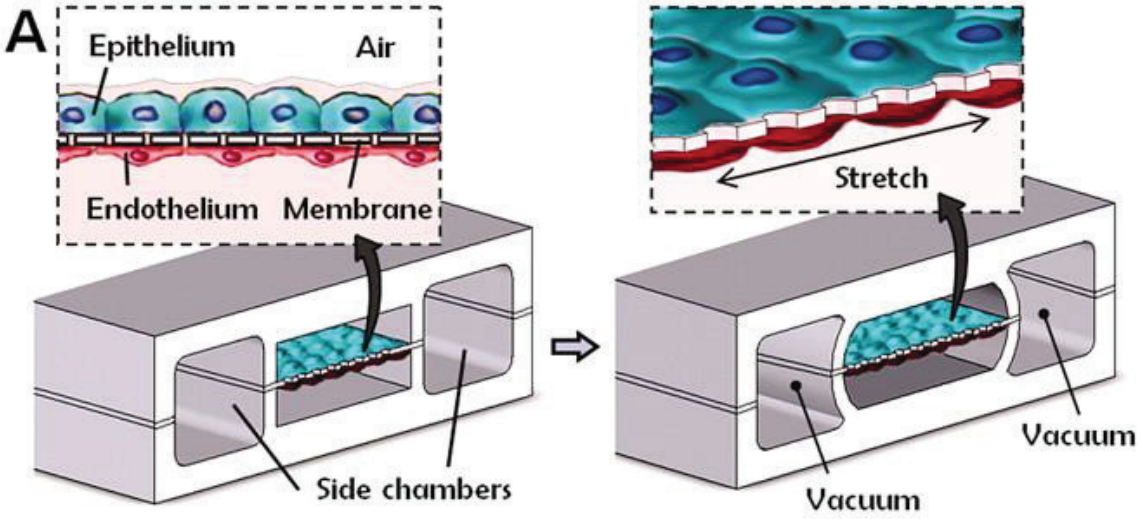
Lung on a chip

- Wyss institute Harvard
- Mimics mechanical and biochemical complexity of lung
- Made from human lung epithelium and endothelial cells cultured either side of a permeable and flexible membrane
- Cyclical mechanical stretching to mimic breathing (via vacuum)
- Flow immune cells in buffer below membrane
- Proposed uses
  - Predict absorption of airborne nanoparticles (enhanced by mechanical stretch)
  - Inflammatory response



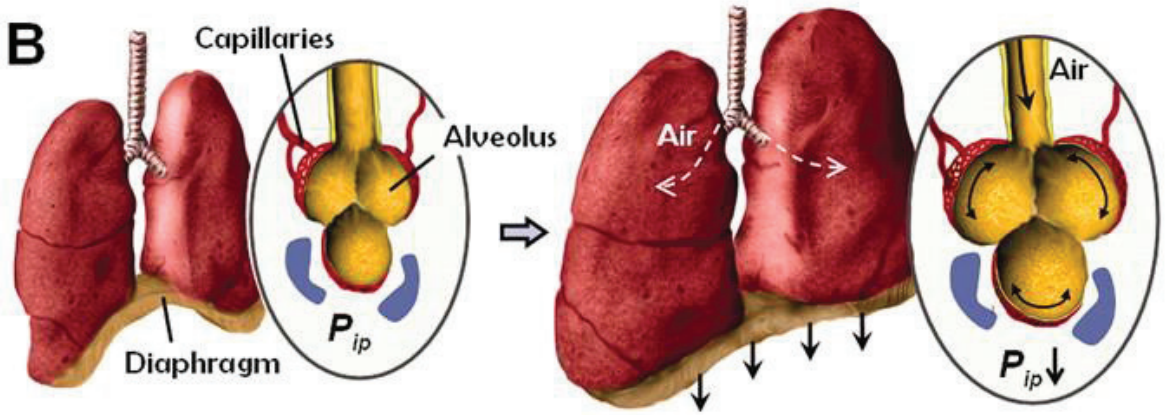
http://wyss.harvard.edu/viewpressrelease/36/living-breathing-human-lungonachip-a-potential-drugtesting-alternative

Wyss - human breathing lung-on-a-chip microdevice.



Membrane

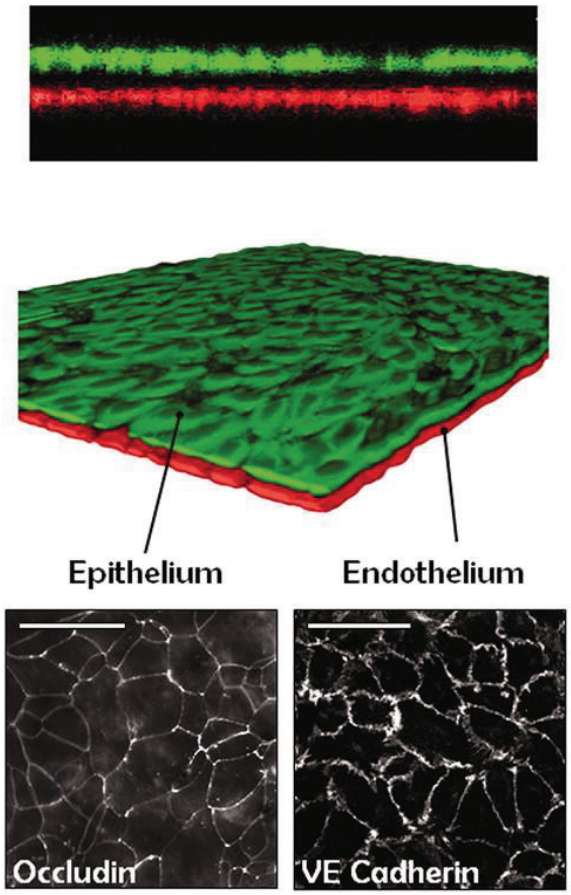
- 10 um thick
- Porous
- Flexible
- Poly(dimethylsiloxane)

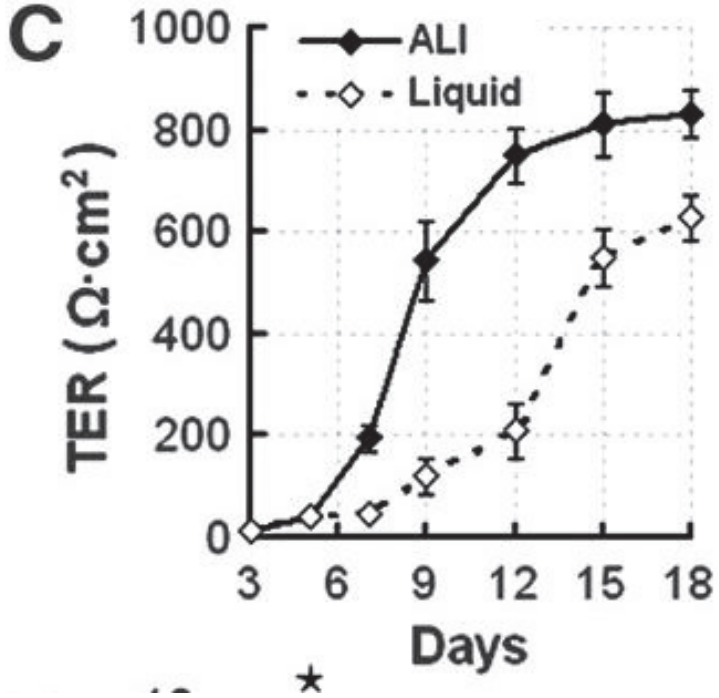


Air upper compartment
Fluid flowing lower compartment

D Huh et al. Science 2010;328:1662-1668

Wyss - human breathing lung-on-a-chip microdevice.





Viable > 2 wks
Surfactant produced

D Huh et al. Science 2010;328:1662-1668

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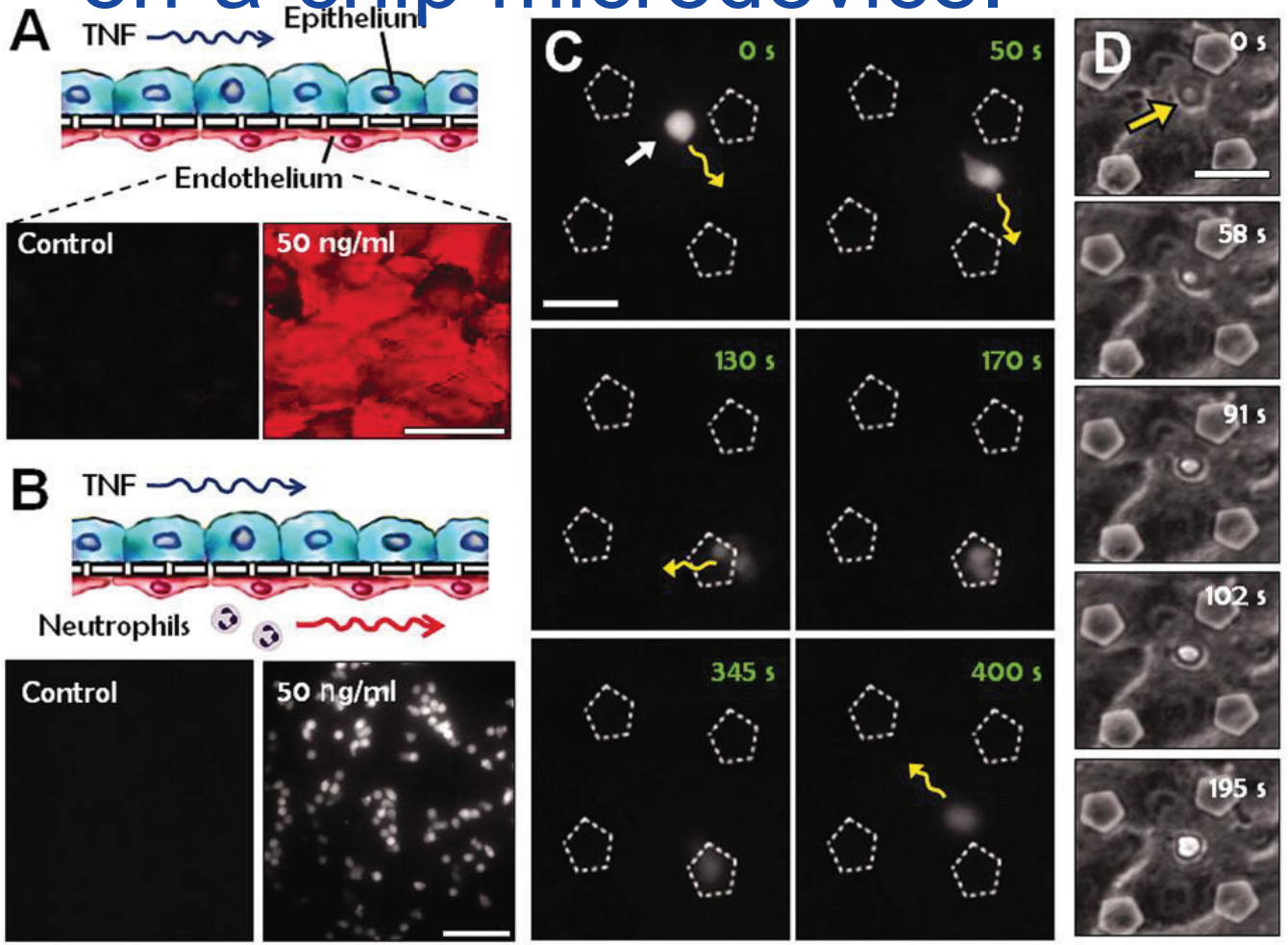
Wyss - human breathing lung-on-a-chip microdevice.



IL8
E.Coli
Silica NP
Inject suspension + aspirate

D Huh et al. Science 2010;328:1662-1668

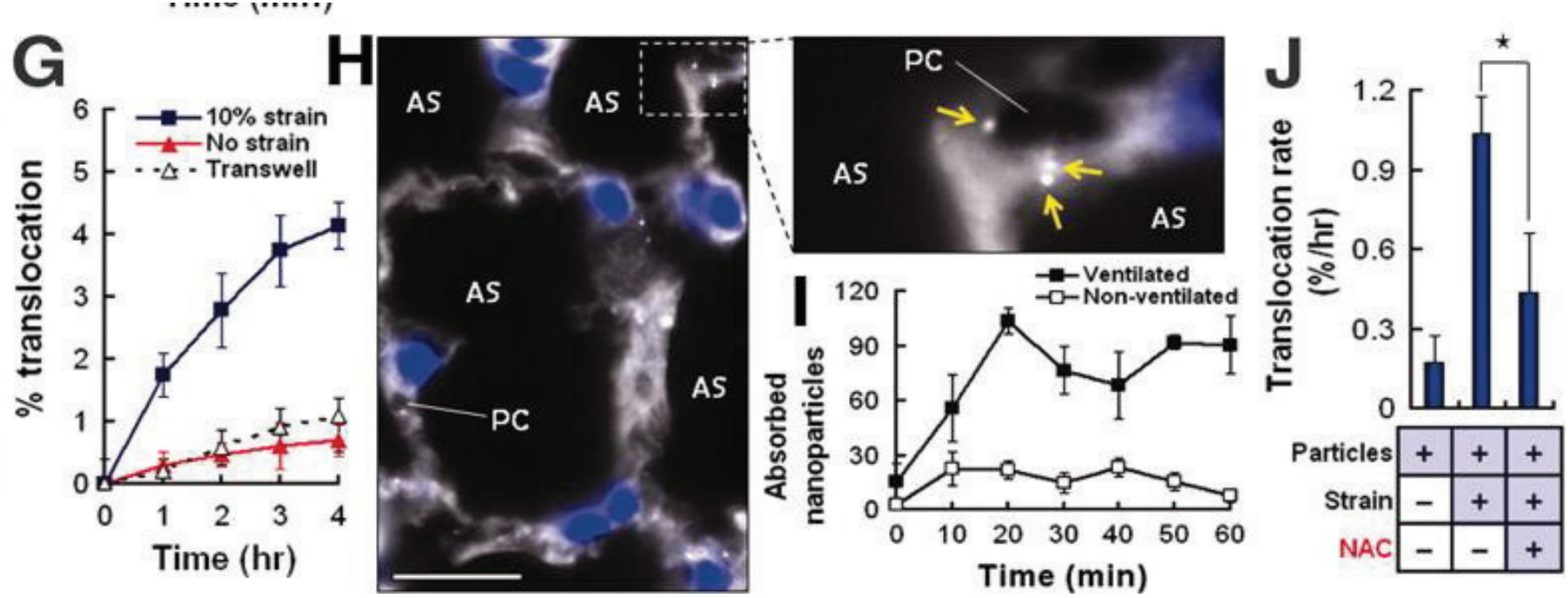
Published by AAAS

Distinct

Science

AAAS

Wyss - human breathing lung-on-a-chip microdevice.



Cyclic mechanical strain accentuates SiO_2 NP translocation, ICAM-1 expression and ROS production (inflammation?)

D Huh et al. Science 2010;328:1662-1668

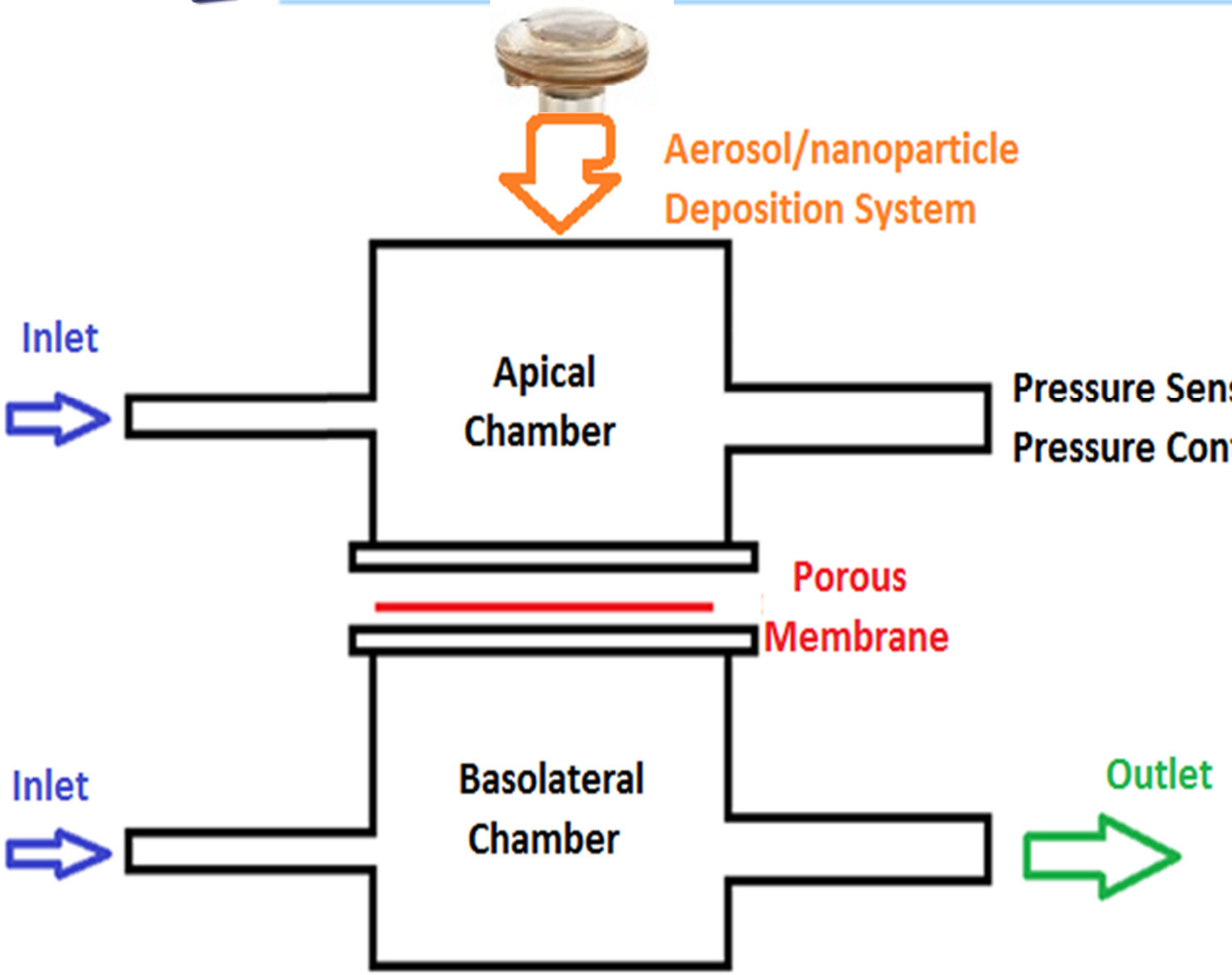
Published by AAAS

Why organ-on-chip won't be here any day soon !

kirkstall

- Air bubbles block flow
- Not enough space to grow physiologically relevant 3D
- Difficult to use soft scaffolds (collagen) (viscosity too high)
- Cannot use hard scaffolds (alvetex/nanofibres/electrospun)
- High surface to volume ratio (absorption problem)
- Too few cells to produce detectable metabolite concentration (high media volume to cell ratio)
- Seeding protocols is tricky (> 1year to train PhD student)
- Millipore PEARL system appears to suffer from non-specific binding of drugs to plastic so active dose is difficult to calculat

MALI Bioreactor



Apical chamber -> air to simulate ***alveolar side***

Basolateral chamber -> liquid (medium) to model ***blood alveolar vassel side***

Cei D.,et al, Proc. XIX Conf Mech in Med.& Biol.2014,

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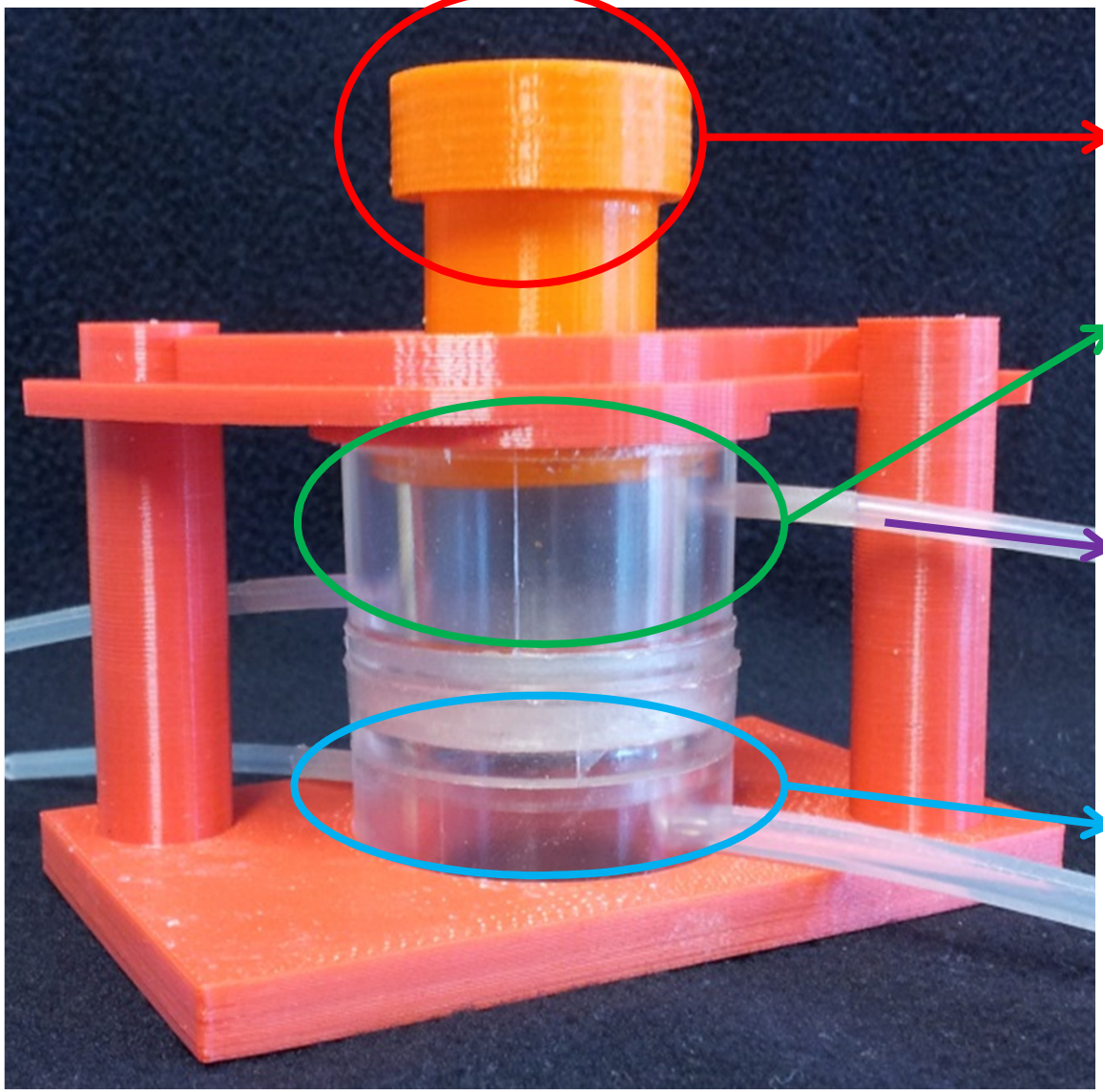
MALI Bioreactor: prototype

Cei et al. Development of a dynamic model of the alveolar interface for the study of aerosol deposition



Nebuliser: provides aerosol cloud (Aeroneb Pro® System)

Apical chamber: filled with air to deposit aerosol on cells and mimic an air-liquid interface

Air supply for membrane activation: with a control system to regulate air injection and remove (membrane vibration)

Basal chamber: with a flowing liquid for nutrient supply and waste remove

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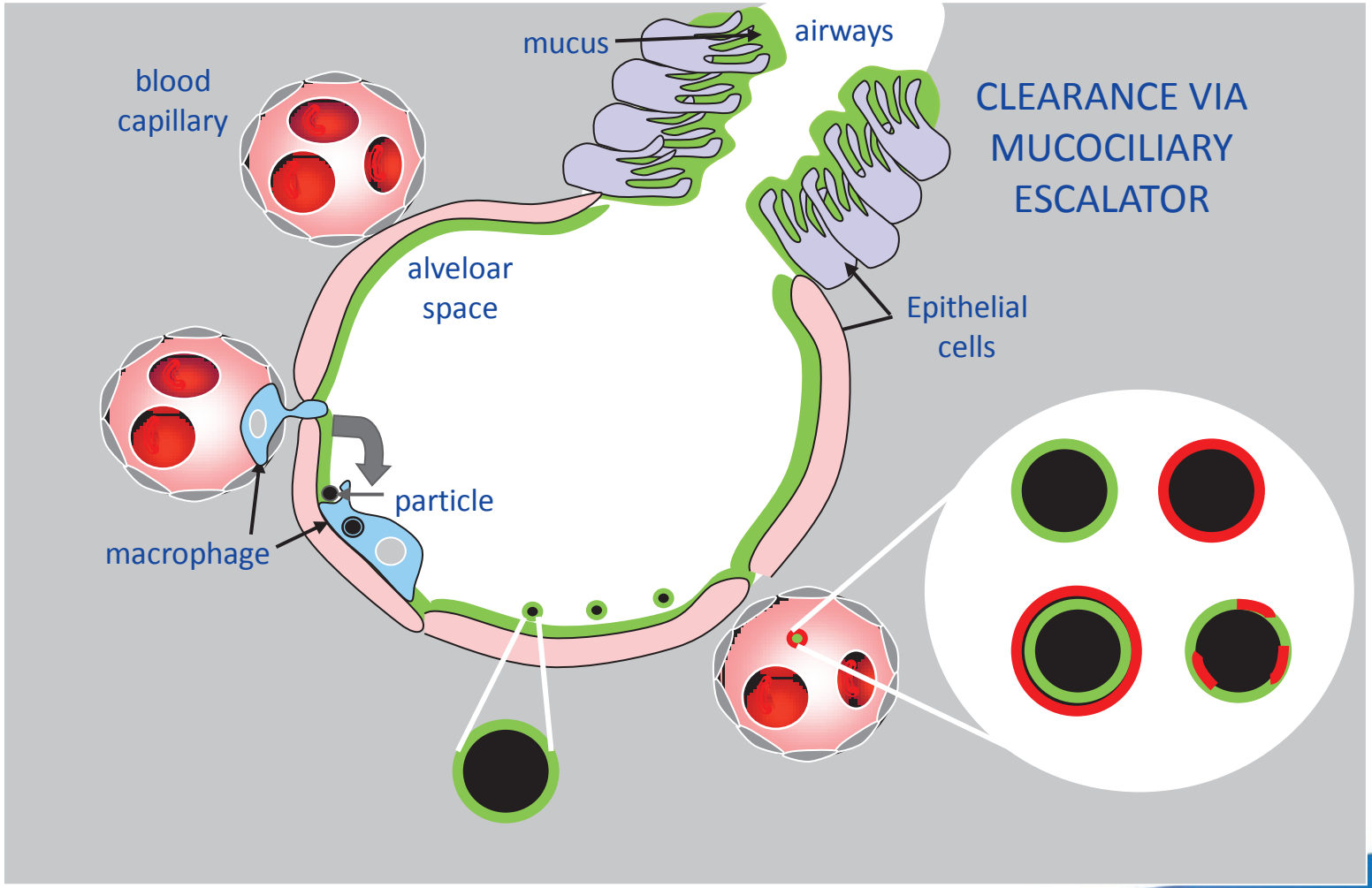
Summary

- A number of options exist to take the newly developed pulmonary NP toxicology model a stage further
  - Microfluidics of different scales
  - Mechanical stress
- Can we take the best of the 2 models described and combine them?
- Do we need to wait for the outcome of the current study before we start to work on such a system?

Manipulate NM to reflect route of entry



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Dose *in vitro* and *in vivo* - lung

Donaldson et al. *Inhal Toxicol* 2008 January;20(1):53-62.

Compared inflammation *in vivo* vs IL8 production by A549 cells *in vitro* and compared dose able to induce effects.

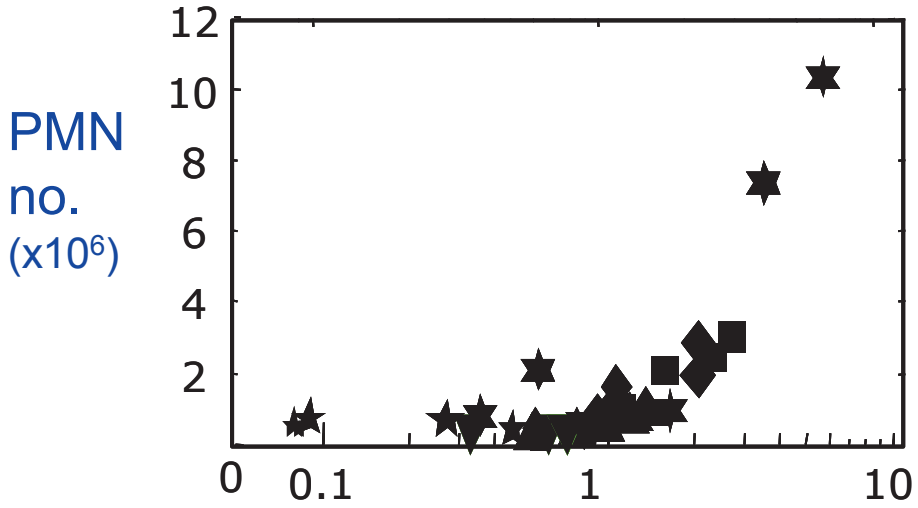
Proximal alveolar region (PAR) of the lung

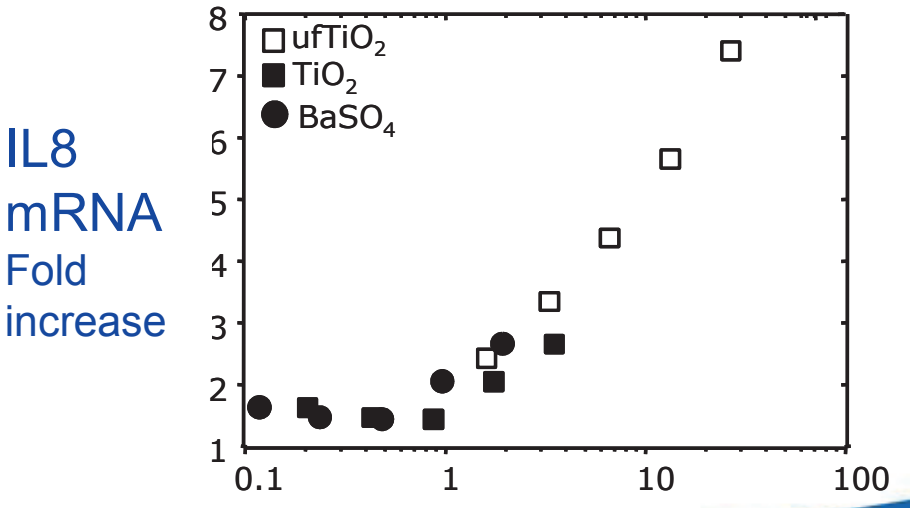
- Terminal bronchioles + alveoli
- Receives high deposition but slow clearance compared to the larger airways
- A few days after exposure residual dose is concentrated in PAR

Dose *in vitro* and *in vivo* - lung

Donaldson et al. *Inhal Toxicol* 2008 January;20(1):53-62.

- Expressed rat lung data as:
  Particle surface area / Proximal alveolar region surface area
- Obtained a threshold value for onset of inflammation of 1 cm^2/cm^2
- IL-8 gene expression by A549 cells *in vitro* dose obtained a threshold of 1 cm^2/cm^2





Understanding Limitations

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Problem: Current RA approaches are heavily dependent on longer term *in vivo* models

OECD Subchronic Inhalation

- Test Guideline 413 (updated 2008)
- 90-day inhalation study
- ‘provide robust data for quantitative inhalation risk assessments’
- Groups of 10 male and 10 female rodents exposed 6 h/day for 5-7 days/week for 90 days (13 weeks)
- 3 or more concentrations of test article, filtered air, vehicle control
- Flexibility – reversibility, BAL, neurological tests, clinical pathology and histopathological evaluations

Why are *in vitro* and *in vivo* studies conducted?

- To work out the physiology, biochemistry, molecular biology and genetics of healthy organisms.
- To work out the cause or mechanisms of disease.
- To identify potential biomarkers of disease for diagnosis.
- To identify potential targets that can be improved to treat disease.
- To test drugs for their efficacy (do they work) and toxicity (are they harmful)
- To test chemicals for their toxicity.
- Reproductive studies to help modify fertility, conception and to induce/prevent pregnancy…..

What are the advantages and disadvantages of using cell culture models?

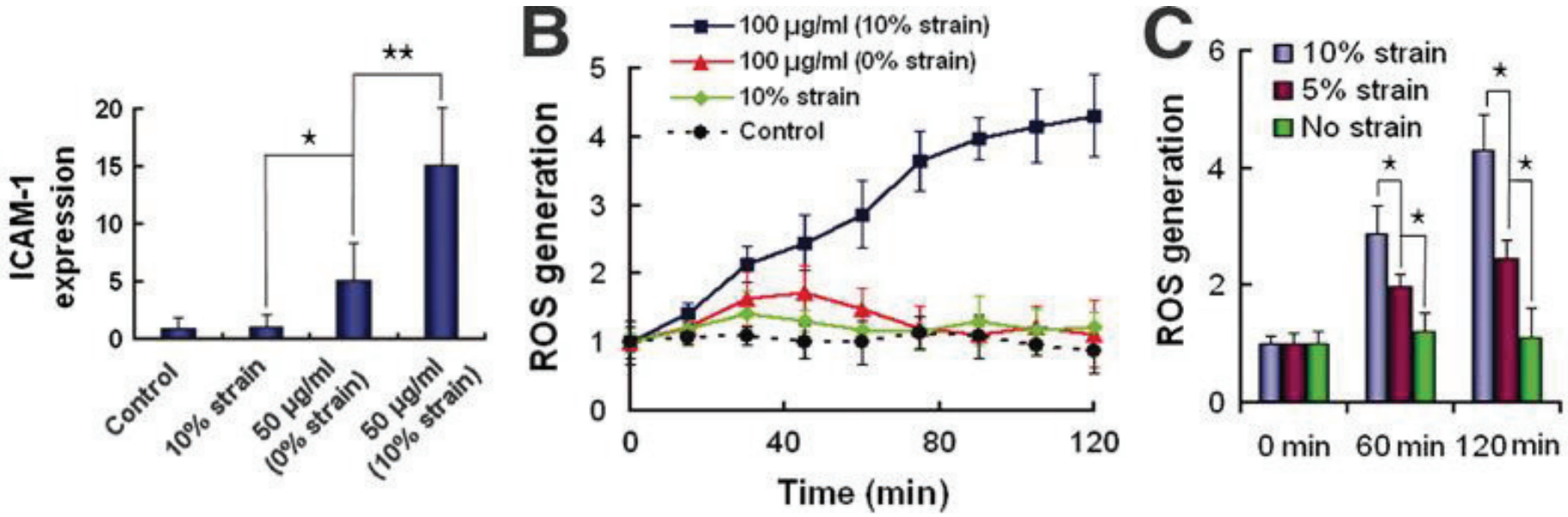
Disadvantages:

- Looking at cells in isolation without communication to other cell types, so response could be incomplete or misleading.
- Very artificial, cell may respond very differently in conditions of culture.
- Cell may change its behaviour over time in culture. E.g. Liver hepatocytes reduce their ability to metabolise toxins

Advantages:

- Ethics – fewer or no animals sacrificed
- Cost is less financially than for *in vivo* studies.
- Can use human cells.
- Can study the same immortalised cells over time and across different laboratories.

Wyss - human breathing lung-on-a-chip microdevice.



D Huh et al. Science 2010;328:1662-1668

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