UNIVERSITÀ DI PISA

Centro 3R

DIPARTIMENTO DI INGEGNERIA DELL'INFORMAZIONE

METODICHE ALTERNATIVE ALLA SPERIMENTAZIONE ANIMALE: LE 3R

Arti Ahluwalia

Research Center E. Piaggio
University of Pisa

Cosa sono le 3R?

Replacement, Reduction, Refinement

Responsible, Rigorous, Reproducible

Ricerca Responsabile=Ricerca di Qualita'

INTERUNIVERSITY CENTER FOR THE PROMOTION OF 3R PRINCIPLES IN TEACHING AND RESEARCH
CENTRO 3R

3R TIMELINE IN EUROPE & ITALY

1959 — "The principle of Human experimental techniques" Russell and Burch

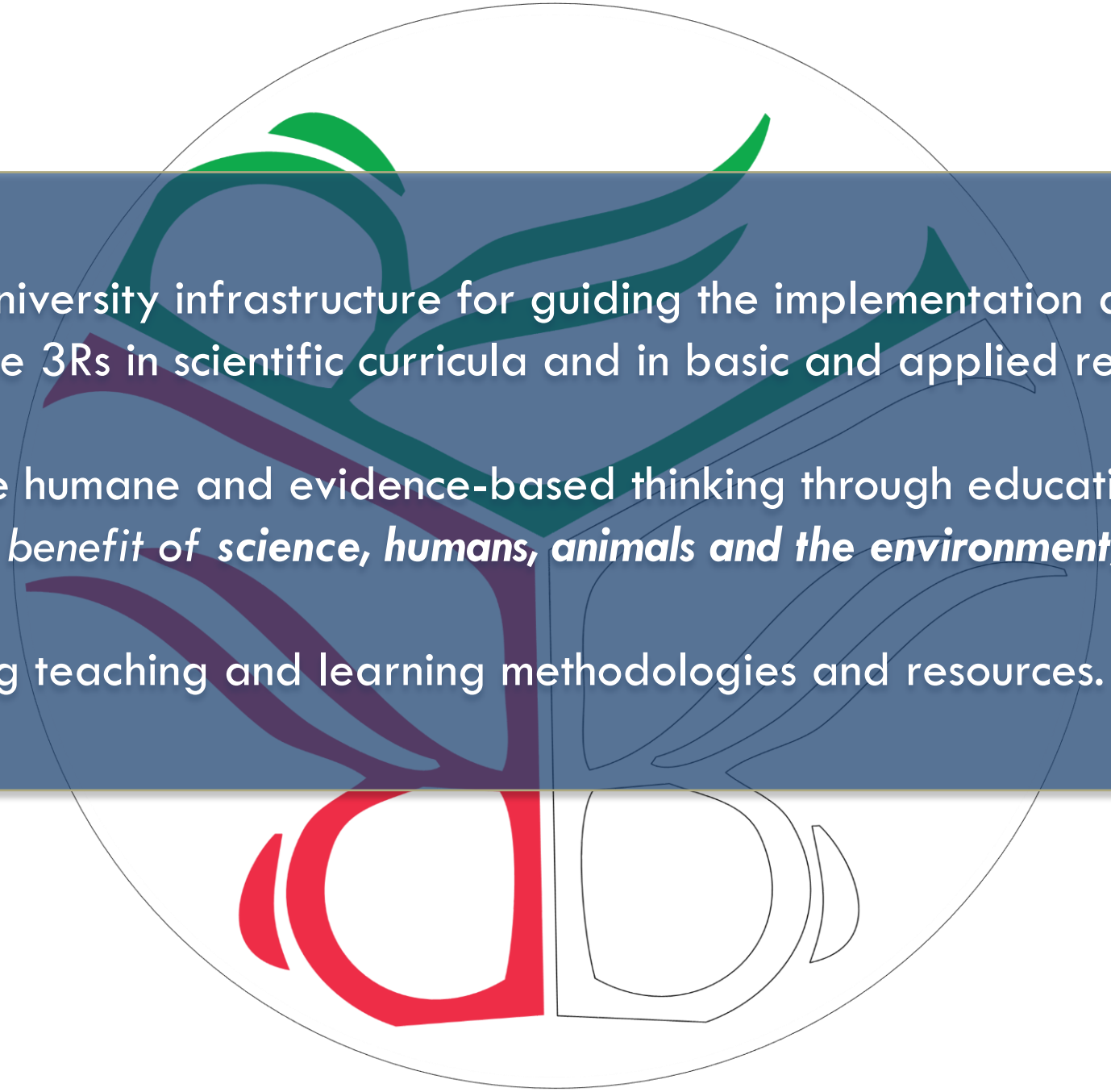
1991 — Foundation of ECVAM, the European Center for the Validation of Alternative methods

2010 — European Directive 2010/63/EU "member states must follow the principle of replacement, reduction and refinement"

2014 — Directive is translated into Italian law: DL 2014/26 on the protection of animals used for scientific purposes

2016 — Working group set up by the Italian Ministry of Health to accelerate the implementation of the directive

2018 — **Centro 3R in Italy!**



Vision: an interuniversity infrastructure for guiding the implementation and integration of the 3Rs in scientific curricula and in basic and applied research;

Mission: promote humane and evidence-based thinking through education and research *for the benefit of* ***science, humans, animals and the environment;***

Strategy: sharing teaching and learning methodologies and resources.

CURRENT MEMBERS

UNIVERSITÀ
DI PISA

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POLITECNICO
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DI PAVIA

CAMPUS BIOMEDICO

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MULTIDISCIPLINARY COMPETENCIES

BIOLOGY

COMPUTATIONAL MODELS

AWBs

BIOMATERIALS, BIOREACTORS & BIOFABRICATION

REGULATION/ LAW /ETHICS

TOXICOLOGY

PHARMACOLOGY

PATHOLOGY

VETERINARY SCIENCE

THE BOARD

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UNITA' OPERATIVA 2

UNITA' OPERATIVA 3

UNITA' OPERATIVA N

...

Achievements

- FROM 2 TO 7 UNIVERSITIES IN 3 YEARS
- OVER 400 MEMBERS
- EXPERT MEMBER OF THE NATIONAL COORDINATION GROUP ON WELFARE OF LAB ANIMALS
- COORDINATION BETWEEN THE NATIONAL CENTER OF REFERENCE/CENTRO 3R/ PARERE
- SECOND ANNUAL MEETING, PRIZES for YOUNG SCIENTISTS
- NEW WEBSITE with SHARED RESOURCES
- EU3RNET MEMBER

Teachig

4 NEW COURSES ON EXPERIMENTAL MODELS BASED ON 3RS IN DEGREE PROGRAMS

- PHARMACY
- BIOLOGY
- BIOMEDICAL ENGINEERING
- MEDICINE
- 2 PHD COURSES

Still to be done

- FUNDING
- ADDING GOOD PRACTICE IN EXPERIMENTAL SCIENCE & USE OF 3RS AS CORE SKILL IN CURRICULA DESCRIPTORS
- A CHAIR?
- MORE UNIVERSITIES NEED TO BE INVOLVED
- 3R CONSULTANTCY

WHAT'S IN A NAME?

Metodi alternativi

NAM- modelli non animali

Specie specifiche

Modelli in vitro

Modelli in silico

Metodi integrati

NAT – non animal technologies

Human based methods

DL. 2014/26. Art 1.

2. E' consentito l'utilizzo degli animali ai fini scientifici o educativi soltanto quando, per ottenere il risultato ricercato, non sia possibile utilizzare altro metodo o una strategia di sperimentazione scientificamente valida, ragionevolmente e praticamente applicabile che non implichi l'impiego di animali vivi.

Art 3.

Scelta dei metodi

1. Non sono autorizzabili le procedure che prevedono l'impiego di animali vivi per le quali esistono altri metodi o strategie di sperimentazione, riconosciute dalla legislazione dell'Unione europea, ovvero prevedono metodi vietati dalla normativa vigente nazionale.

Dove li trovo?

https://www.centro3r.it/it/articoli-e-risorse-web

Siti e documenti di riferimento

European Union Reference Laboratory for alternatives to animal testing - EURL ECVAM. Il sito e' ricco di informazione sulle 3R, particolarmente nell'ambito della tossicologia.

Le linee guida dell'OECD per il test delle sostanze chimiche (TG) sono la raccolta dei metodi più rilevanti concordati a livello internazionale per identificare e caratterizzare i potenziali pericoli di nuovi ed esistenti sostanze chimiche e preparati / miscele chimiche. Di particolare interesse sono le linee guide per i metodi in vitro: **Guidance Document on Good In Vitro Method Practices (GIVIMP).**

Il sito del **Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio** presso l'IZSLER a Brescia contiene diversi link e risorse utili.

Check list delle linee guide PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) in italiano.

Esistono numerosi articoli e libri di riferimento sulle 3R, sul benessere animale e sui metodi alternativi.

Libri e testi di riferimento

1. The Principles of Humane Experimental Technique. W.M.S. Russell and R.L. Burch, London, Methun, 1959. Disponible online.

Il testo classico che definisce le 3R per la prima volta

2. Animal Experimentation: Working Towards a Paradigm Change. Human-Animal Studies. Eds. K. Hermann and K. Jayne, Vol. 22 Brill, 2019. Disponibile online.

Ricco di informazioni e abbastanza recente da contenere aspetti dello stato dell'arte

3. Traduzione dall'inglese dell'articolo "Russell and Burch's 3Rs Then and Now: The Need for Clarity in Definition and Purpose", Journal of the American Association for Laboratory Animal Science, 54:2, 2015, pp. 120–132. Link.

A cura di Ilaria Rizzato, Università di Genova

4. "What is exactly the 'N' in cell culture and animal experiments?", Lazic ES., Clarke-Williams CJ., Munafò MR., PlosONE, April 2018, https://doi.org/10.1371/journal.pbio.2005282

Questo articolo discute il significato del numero N di esperimenti in vivo e in vitro da condurre in modo da overe risultati significativi.

5. PREPARE: guidelines for planning animal research and testing. Smith AJ., Clutton RE, Lilley. E...https://doi.org/10.1177/0023677217724823

*L'articolo descrive le linee guide progettate per essere applicabili alla gestione delle strutture che ospitano animali e la gestione e formulazione di esperimenti che coinvolgono l'uso di animali. Il checklist e' scariabile nella sezione **siti e documenti di riferimento**.*

Riviste

JRC Summer School on "Non-animal Approaches in Science: The Three R...evolution"

La prossima edizione della JRC Summer School on "Non-animal Approaches in Science" organizzata da

Seminario Online Benessere Animale 2020L'

L'Università di Pisa organizza un interessante ciclo di seminari online nei mesi di ottobre, nove

Scuola ESTIV 2021

La European Society of Toxicology In Vitro (ESTIV) è lieta di annunciare che offrirà tre travel g

2nd Centro3R Annual Meeting: 3Rs in Italian Universities

Masquerade

Go

Enter the username to masquerade as.

LINEE GUIDA ARRIVE

Traduzione Ufficale Centro 3R

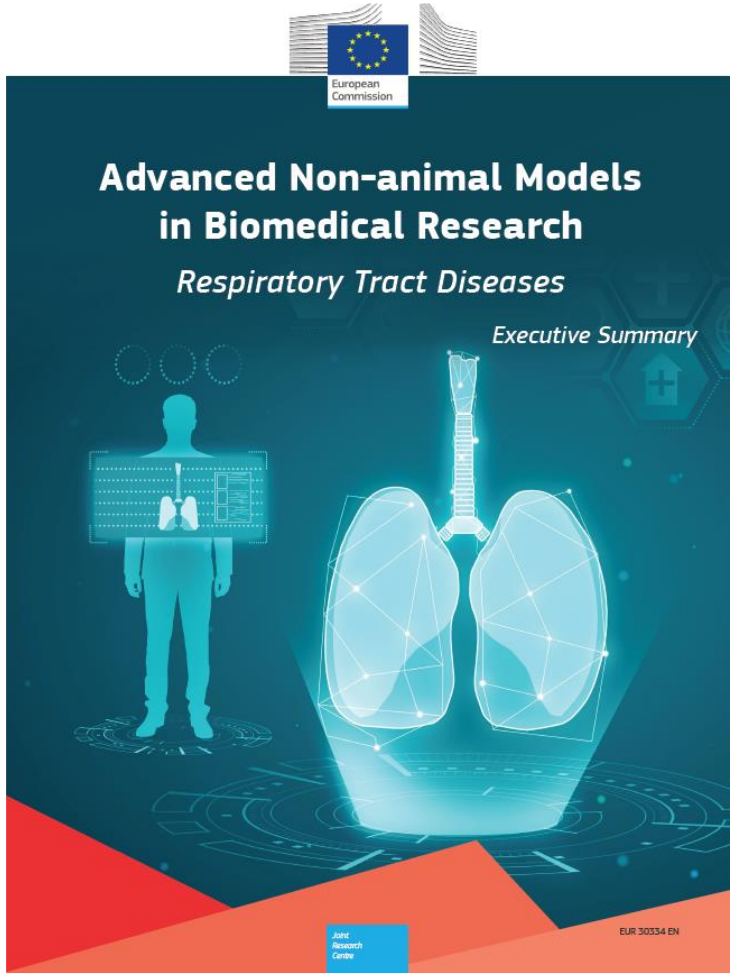
I 10 punti essenziali
Questi sono gli elementi minimi per poter valutare l'affidabilità dei risultati.

1. Impostazione dello studio
2. Numerosità del campione
3. Criteri di inclusione e di esclusione4
4. Randomizzazione
5. Procedure in cieco
6. Misurazione dei risultati
7. Metodi statistici
8. Animali da esperimento
9. Procedure sperimentali
10. Risultati
11. Abstract
12. Background
13. Obiettivi
14. Dichiarazione di eticità
15. Stabulazione e allevamento
16. Cura e monitoraggio degli animali
17. Interpretazione / implicazioni scientifiche
18. Generalizzabilità / traslazione
19. Registrazione del protocollo
20. Accesso ai dati
21. Dichiarazione degli interessi

Linee guida ARRIVE 2.0: line guida aggiornate per le relazioni scientifiche nella ricerca animale. Pubblicate per la prima volta su PLOS Biology, luglio 2020.
Traduzione Ufficiale a cura del Centro 3R, disponibile tra poco sui siti del Centro 3R, NC3R, Norecopa, JRC.

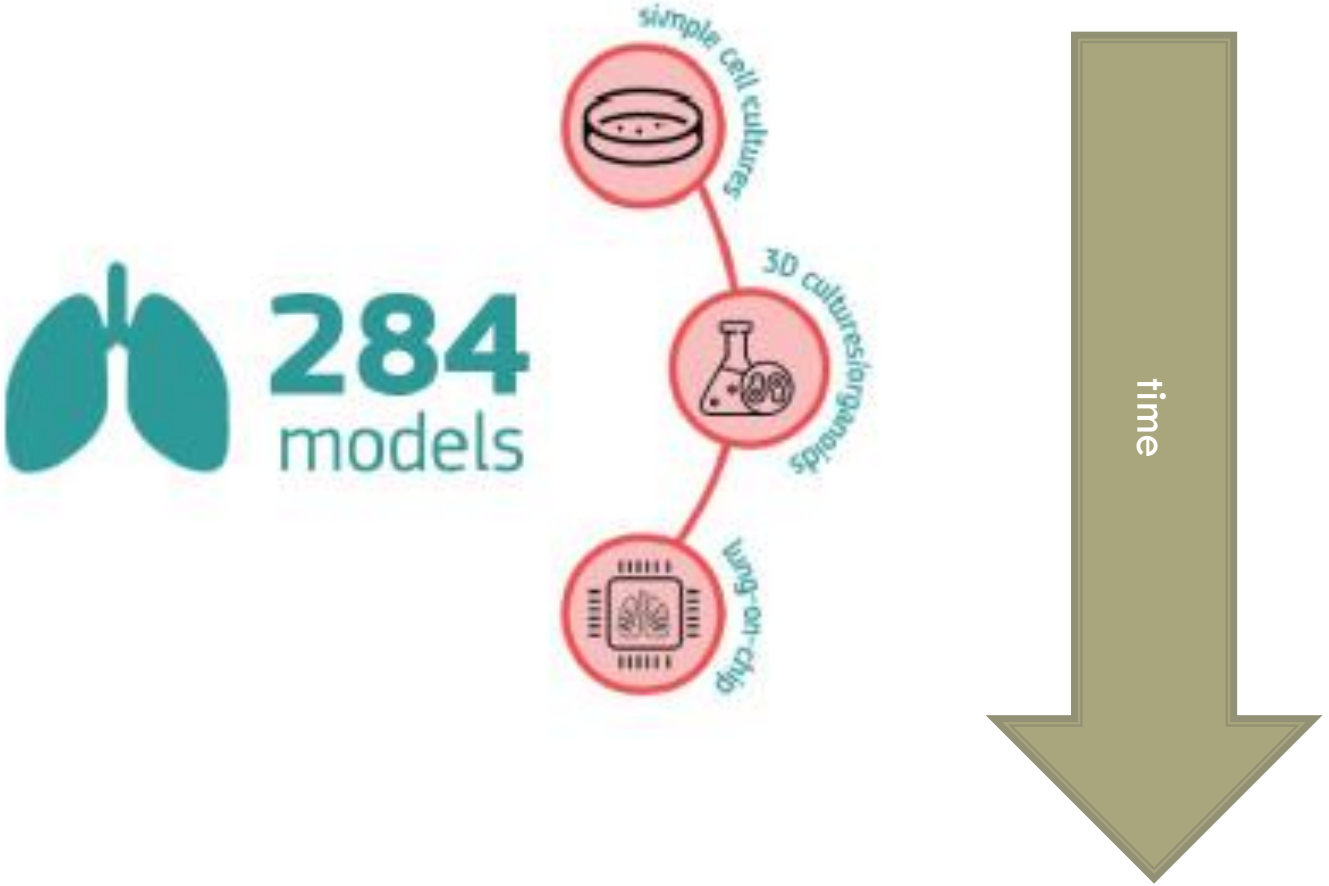
Il sito JRC

https://ec.europa.eu/jrc/en/publication/advanced-non-animal-models-biomedical-research



Respiratory tract diseases

J., Marshall, L., Adcock, I., Novotny, T., Nic, M., Dibusz, K. and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases*, EUR 30334 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-21380-2, doi:10.2760/725821, JRC118161.



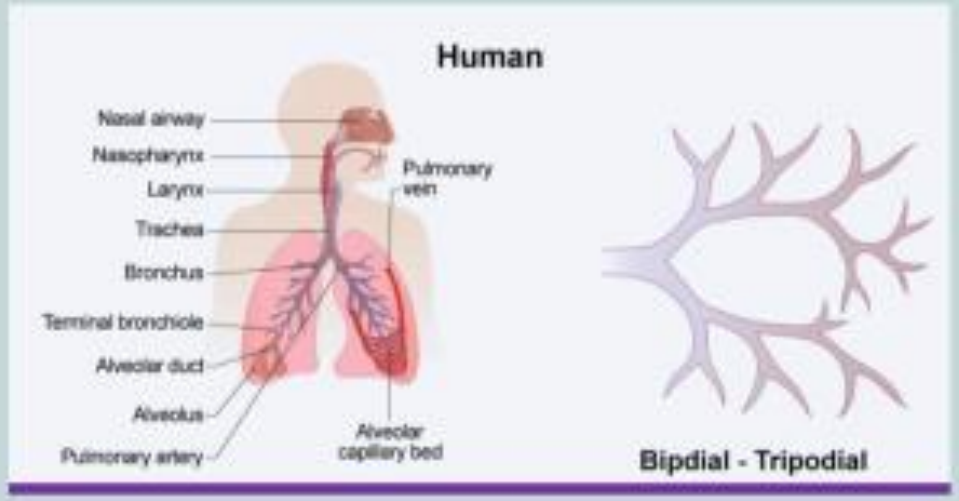
Background

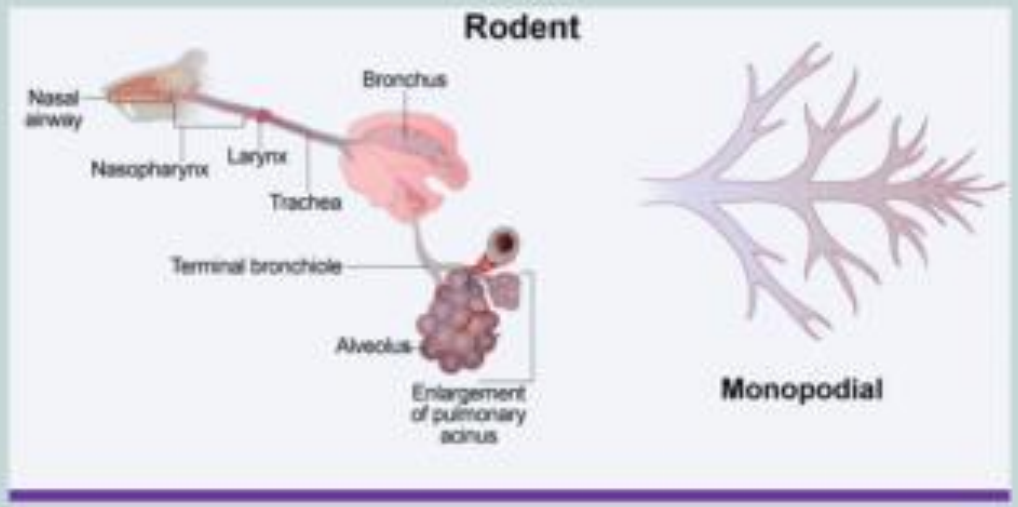
Malattie polmonari : 255 M persone con asma.

Non e' replicabile in topi o ratti

251 M persone con COPD

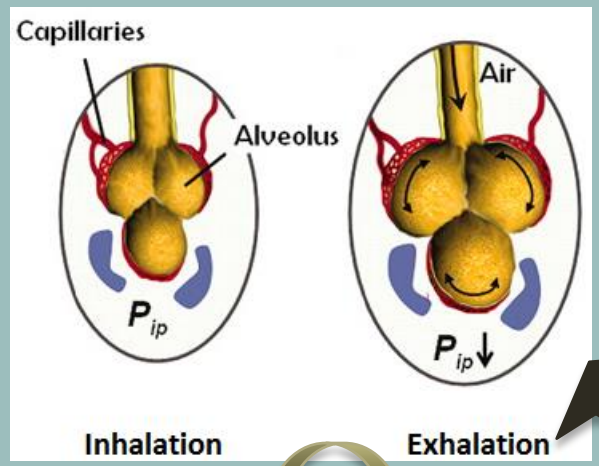
Modelli non-mammiferi poco usati per via delle differenti strutture polmonari



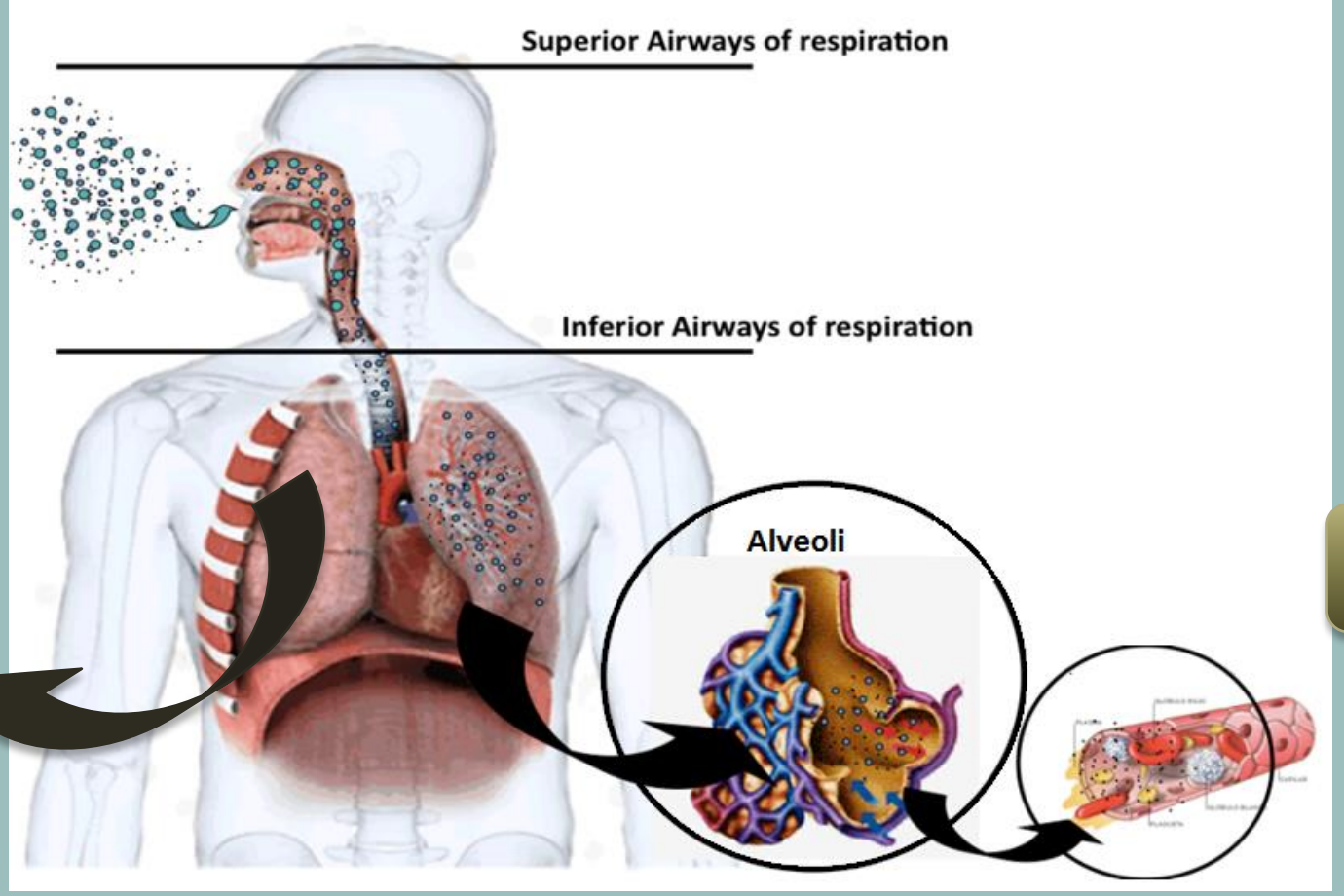


MORPHOLOGICAL DIFFERENCES BETWEEN RODENT AND HUMAN AIRWAYS

A. R. Kolli, A. K. Kuczaj, F. Martin, A. W. Hayes, M. C. Peitsch & J. Hoeng (2020): Bridging inhaled aerosol dosimetry to physiologically based pharmacokinetic modeling for toxicological assessment: nicotine delivery systems and beyond, Critical Reviews in Toxicology, DOI: 10.1080/10408444.2019.1692780



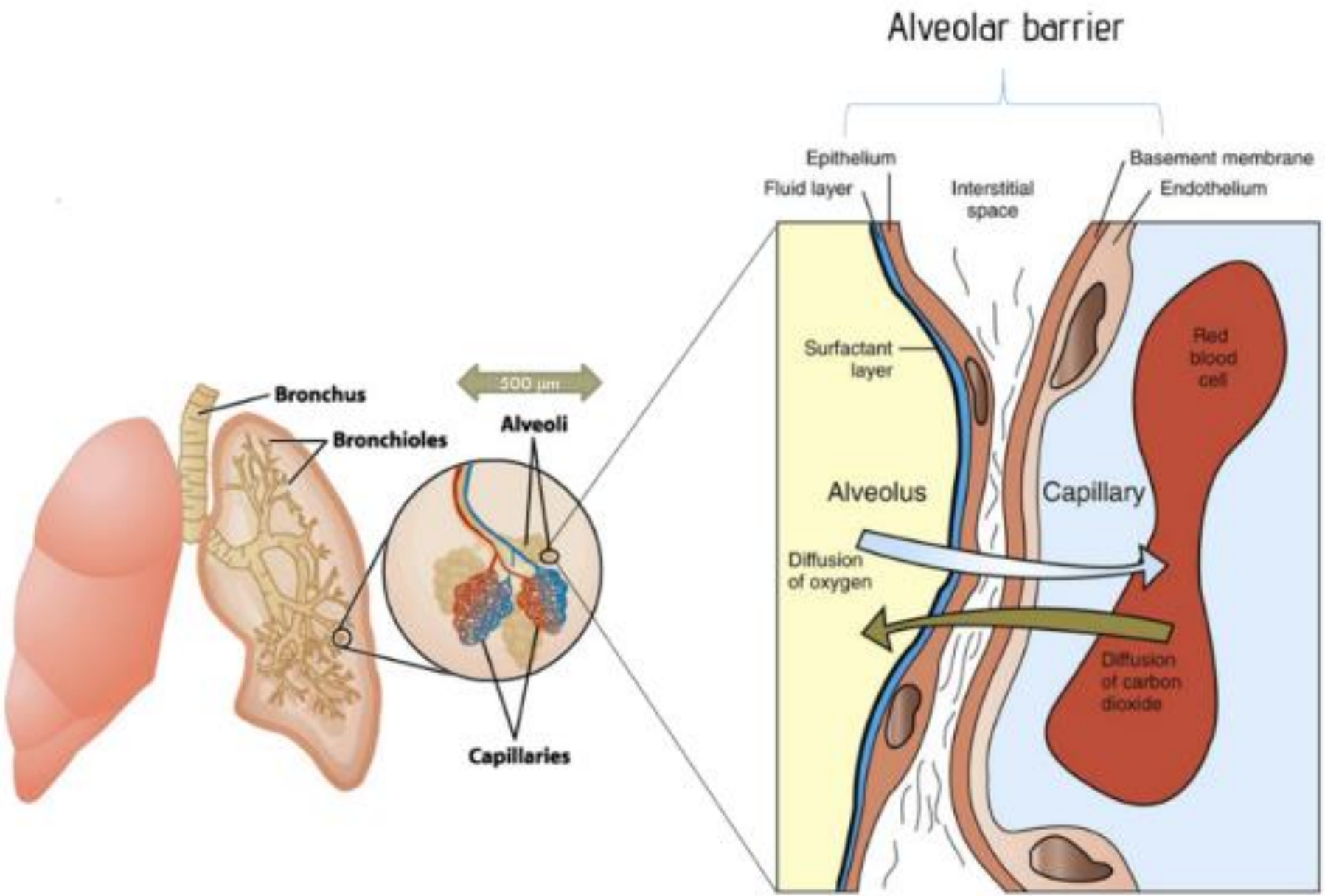
Breathing



Aerosol Deposition

MODELLING HUMAN LUNGS

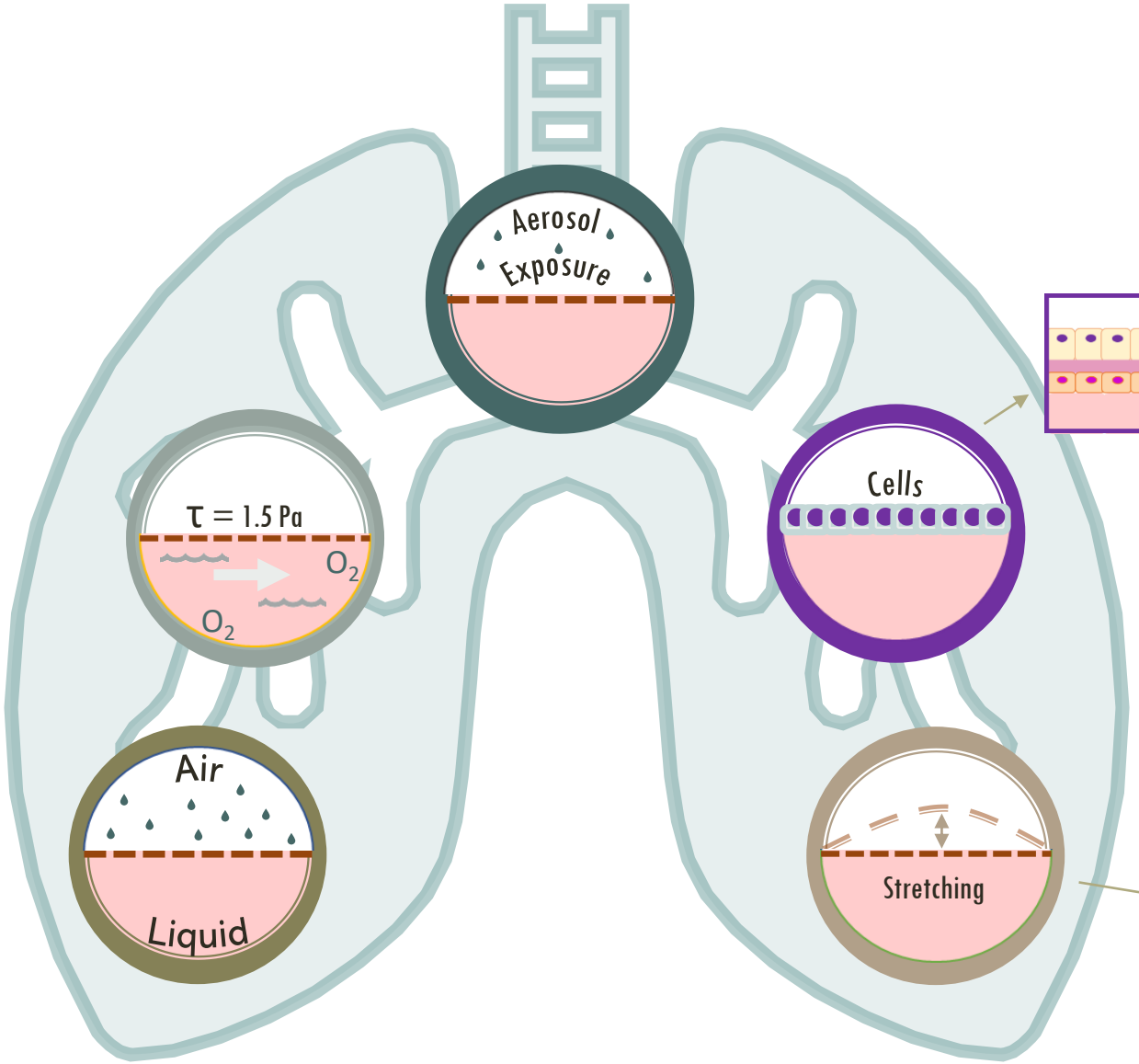
Viral infections	Lung cancer
Smoke	COPD
Medicines	Asthma
Pollutants	Sports
Respiratory stress syndrome	



How do nano-droplets interact with the lungs?

Anatomy of the alveolar interface

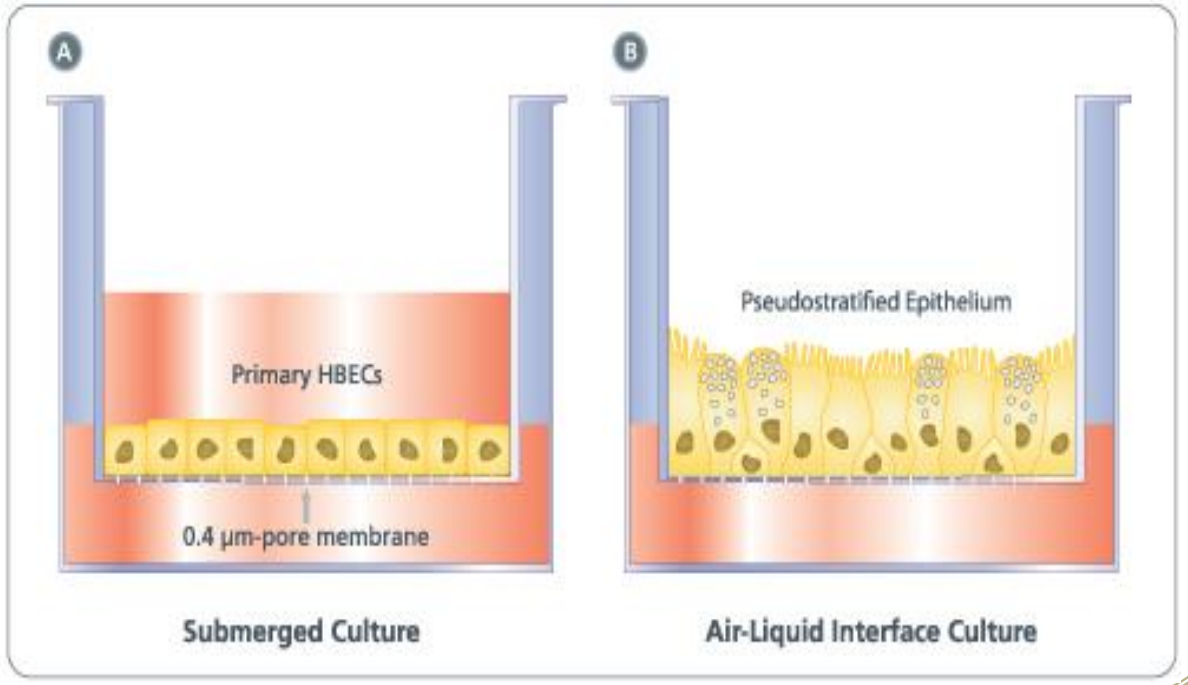
Key elements of the alveolar interface



Air-blood barrier thickness
≈ 0.6 µm

5–12% strain
at 0.2 Hz
(20% pathological)

Petri dishes or Multiwell (transwell) plate: The Air Liquid Interface (ALI) culture



LUNG *IN VITRO* MODELS

Other models: organoids, spheroids, lung on a chip

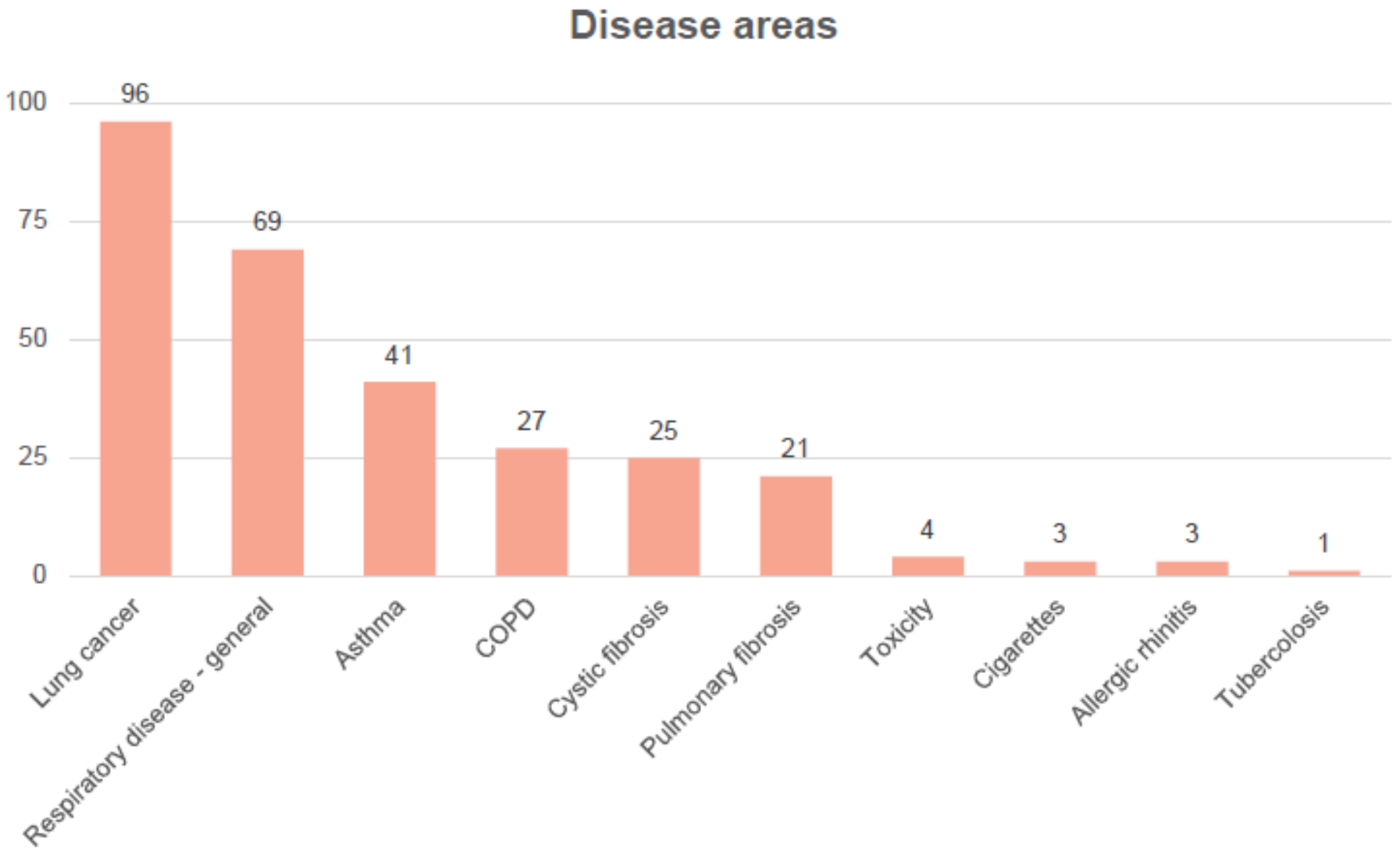


Figure 3: Number of unique non-animal models identified and extracted per disease area.

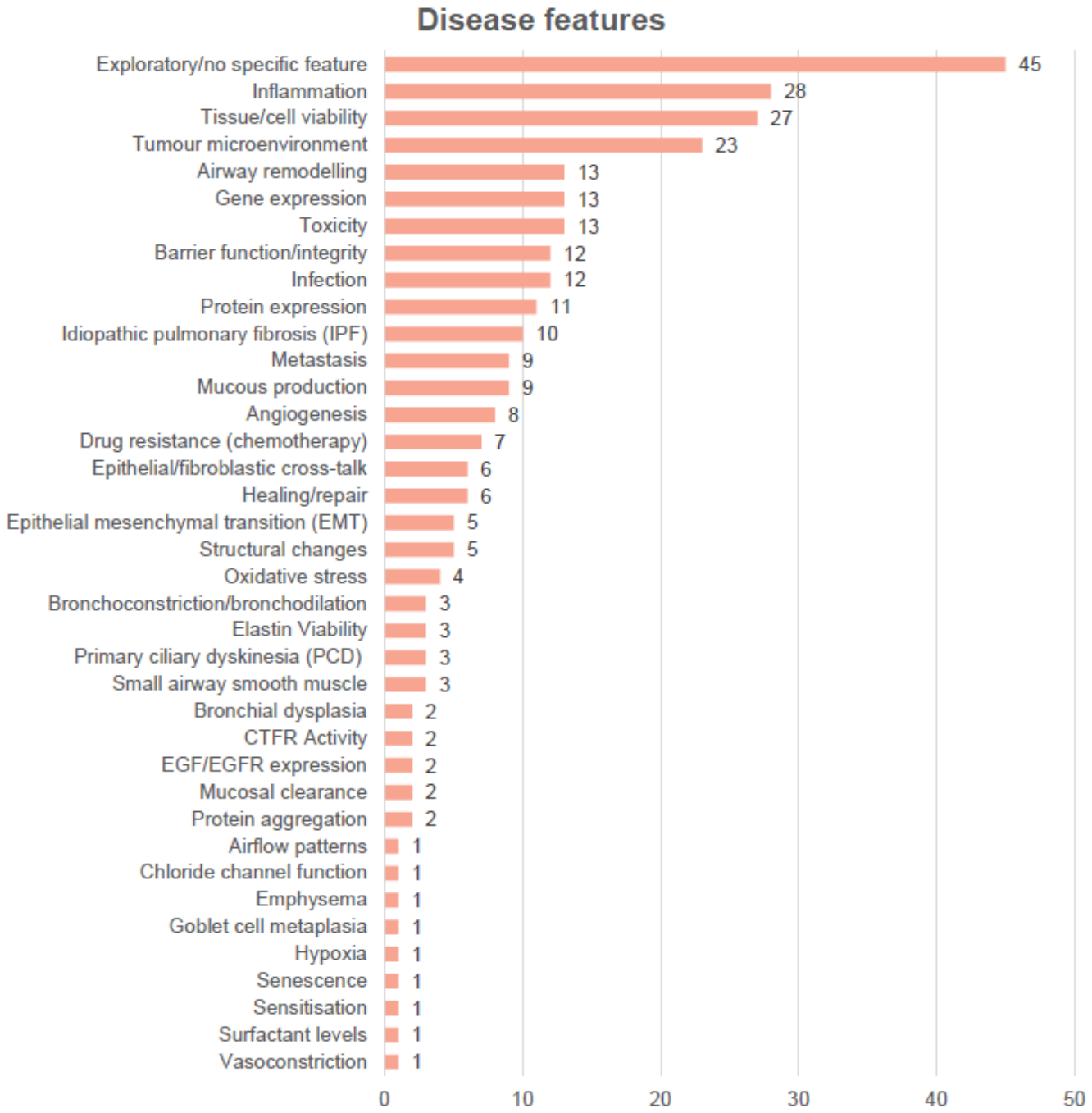


Figure 4: Number of unique models identified and extracted per disease feature.

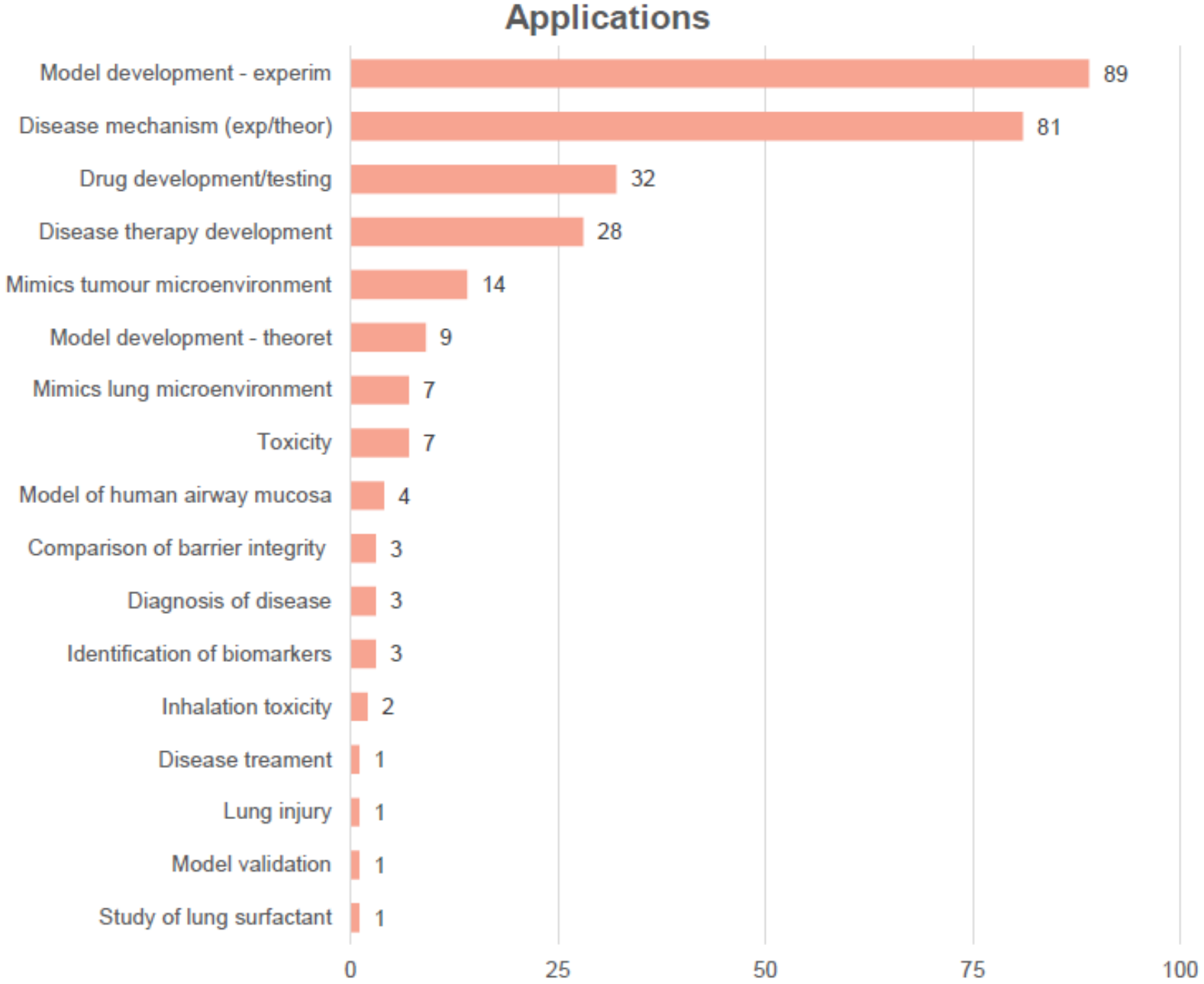


Figure 7: Number of unique models identified and extracted per application.


Protein expression 47
Cytokine release 45
Gene expression 45
Other 43
Tissue/cell viability 41
mRNA expression 34
Immunohistochemistry/immunofluorescence 31
Imaging 28
Trans epithelial electrical resistance (TEER) 24
Inflammatory mediators 15
Metabolism 15
Migration and invasion 15
Cillia beating frequency 10
Cellular crosstalk 8
CTFR activity 8
Morphology 8
Vascularisation 7
Apoptosis 6
Cell/tissue morphology 6
Cell proliferation 5
Mucous production 5
Bioelectric properties 4
Cell/tissue contraction 4
Chemotaxis 4
Airway remodelling 3
Cell migration 3
Mucociliary clearance 3
Epithelial mesenchymal transition (EMT) 3
Airway surface liquid (ASL) volume 2
Cytotoxicity 2
Infection 2
Reactive oxygen species (ROS) 2
Repair 2

ENDPOINTS

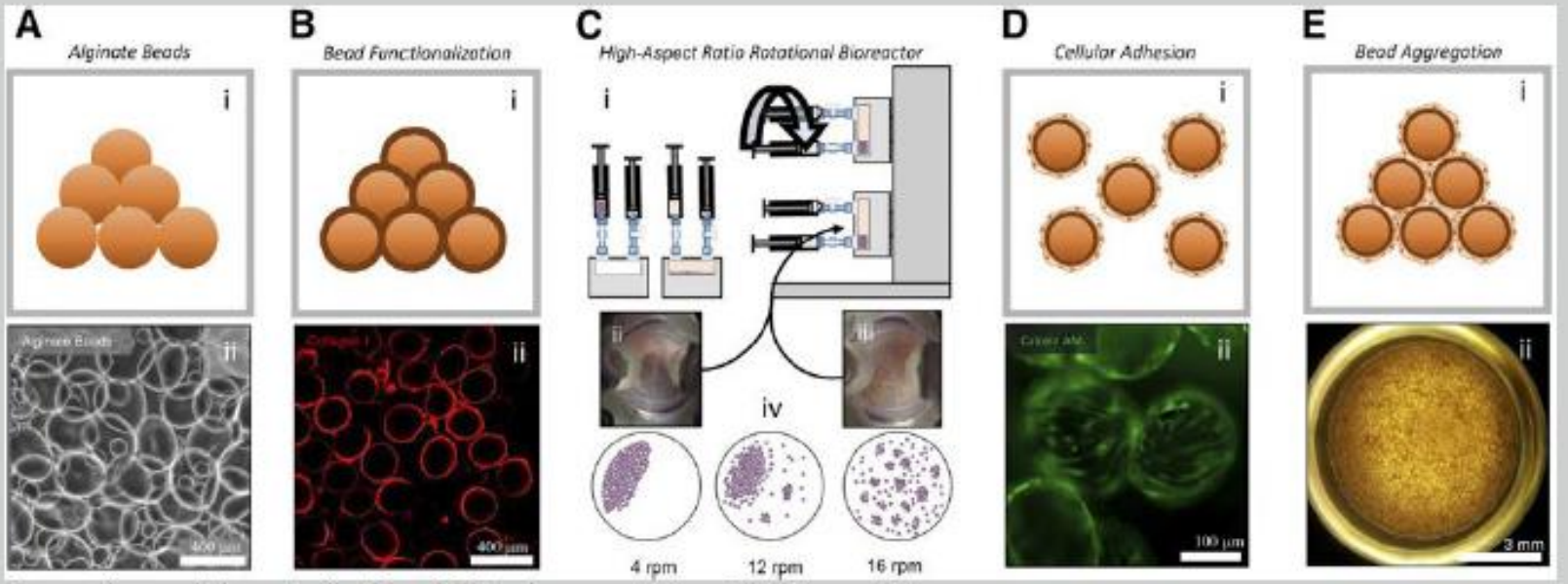
Personalised disease modelling through iPSC-derived mesenchymal cells grown into organoids

There are currently several methods to develop tissue-engineered organoids that replicate the organ's functionality. An important advantage is their potential for high throughput drug screening to identify targeted therapies

Wilkinson *et al.* (2016) generated human lung tissue containing self-assembled multiple lung cell types that allow cell-cell contact and recapitulation of the lung microenvironment.

The organoids formed through the agglomeration of cell-coated alginate beads either in a bioreactor or in a 96-well plate format and remained viable for 2 weeks without degradation.

This system has been found suitable with any cell types including iPSC-derived mesenchymal cells offering a great potential for use in personalised medicine or disease modelling and drug discovery.



Generation and characterisation of 3D pulmonary organoids.

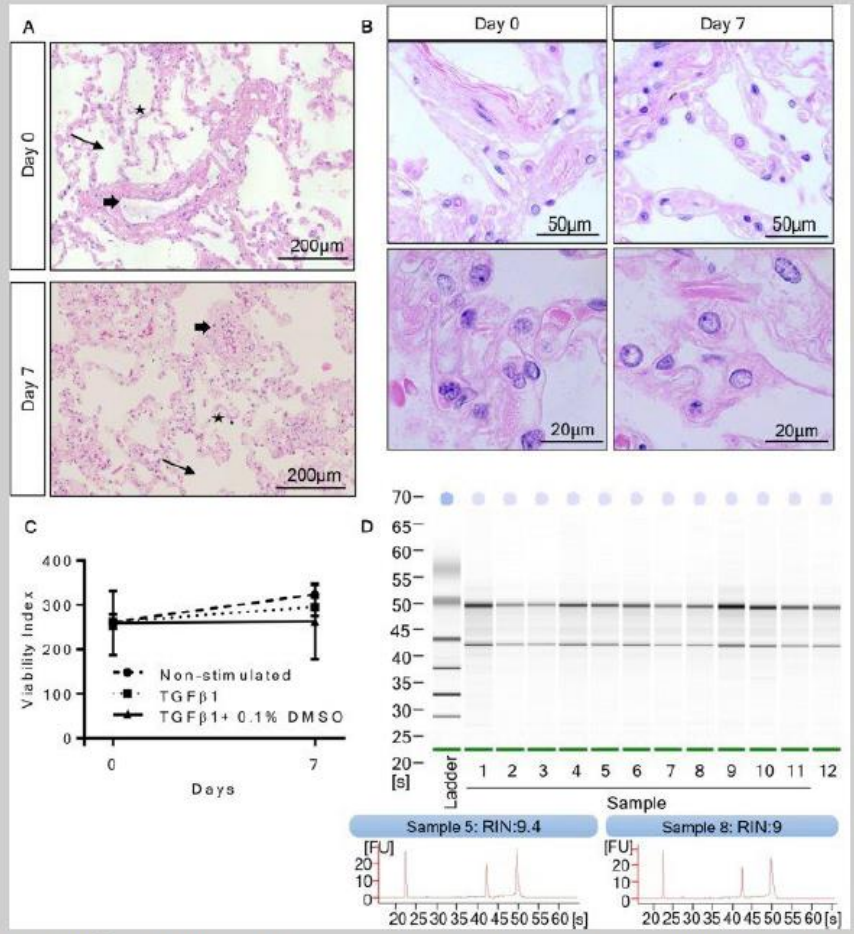
Ex vivo human lung parenchymal model of fibrogenesis

Pulmonary fibrosis is a severe respiratory disease in which scars are formed in the lung tissues, as a result of the accumulation of excess fibrous connective tissue.

Roach *et al.* (2018) have used *ex vivo* human tissue obtained from lung of patients undergoing lung resection for carcinoma to investigate their hypothesis that TGFβ1-induced pro-fibrotic structural responses are inhibited by a selective KCa3.1 blocker.

This model is based on *ex vivo* human lung parenchyma cultured for 7 days in serum-free medium containing TGFβ1 and essential nutrients.

By examining nuclear morphology, tissue necrosis, metabolic activity and RNA quality, the authors assessed the lung tissue viability and response.

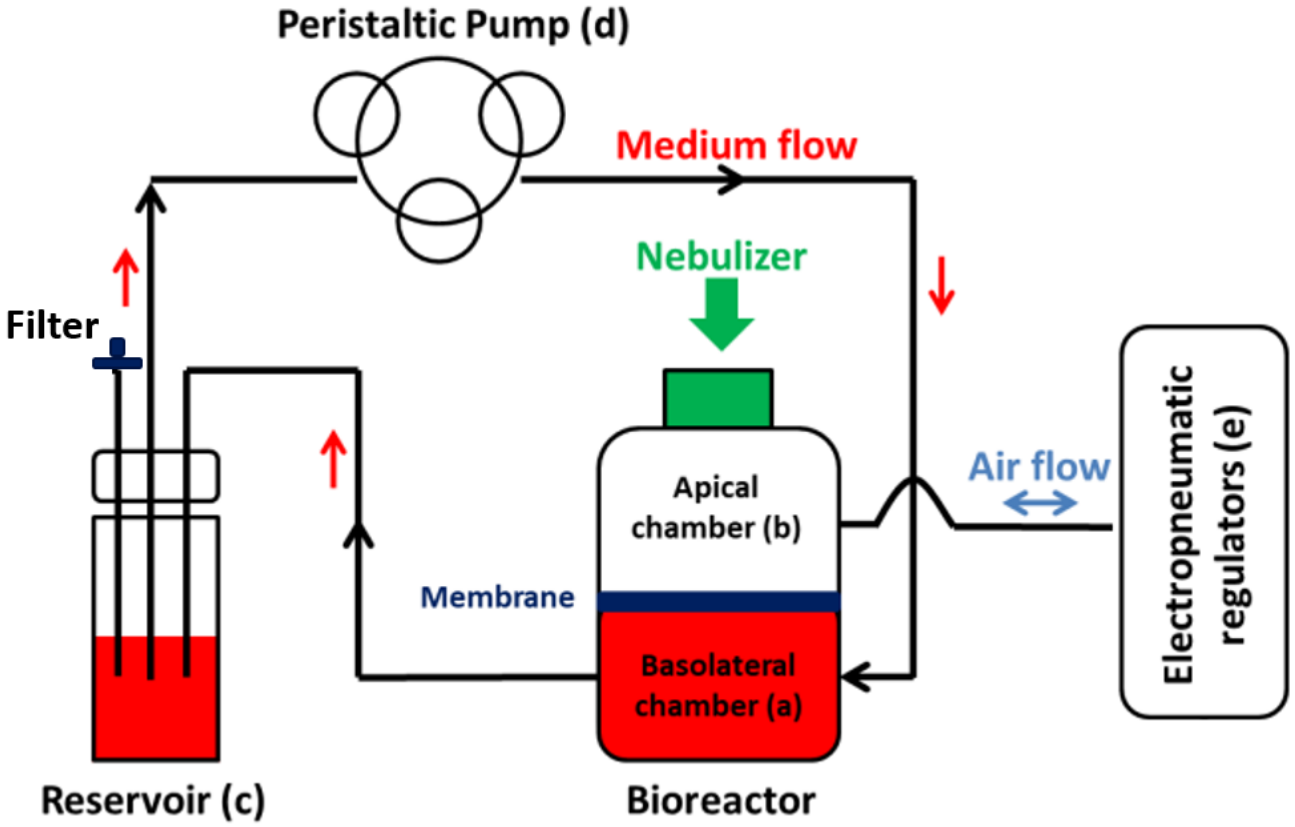


Magnified H&E-stained images of tissue embedded on day 0 or after culture for 7 days in serum-free medium form the same donor shows preserved nuclear morphology with no sign of nuclear fragmentation.

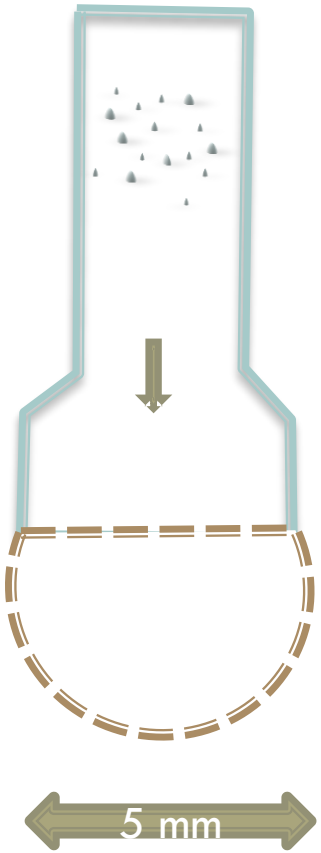
MALI System

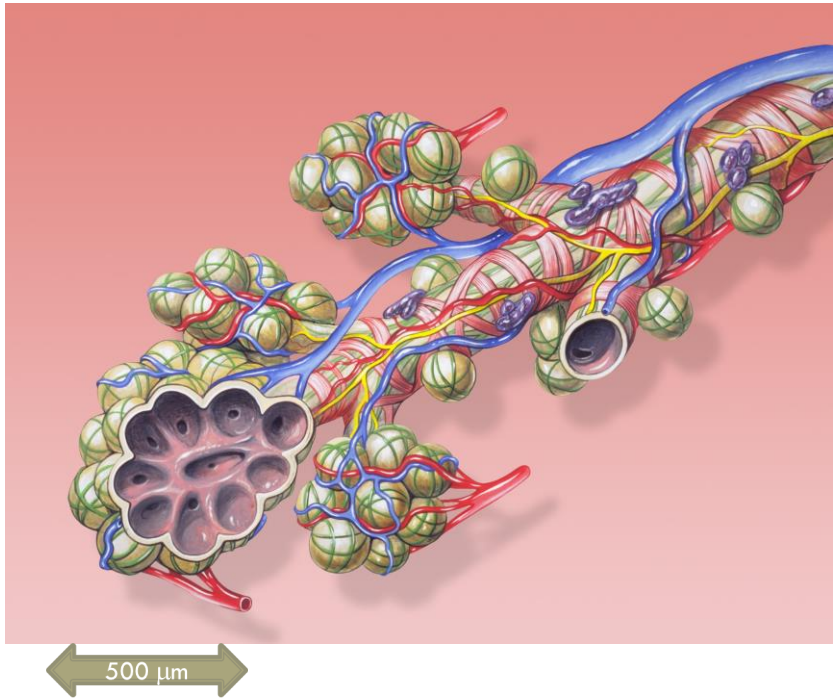
Moving Air-Liquid Interface

An **air-liquid interface (ALI)** bioreactor with a **mobile elastic membrane** to simulate physiological lung stretching *and* an integrated nebulizer for aerosol exposure



MALI ALVEOLAR MODEL: AEROSOL DEPOSITION



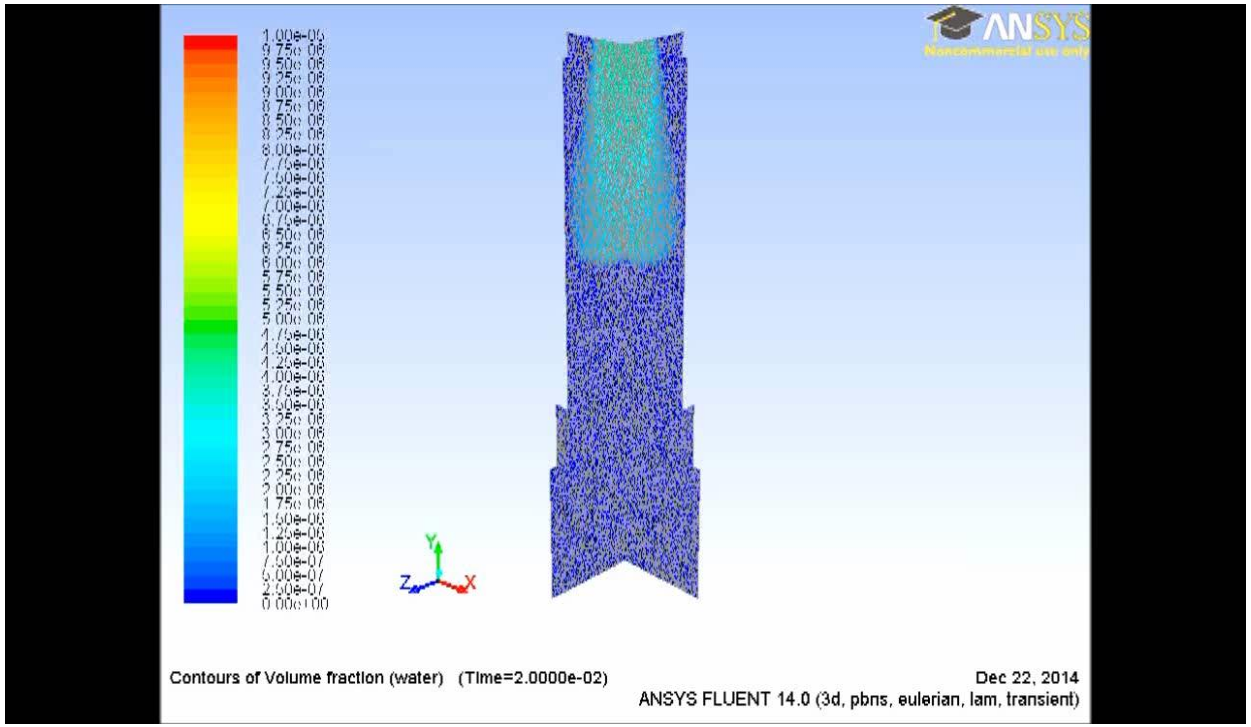


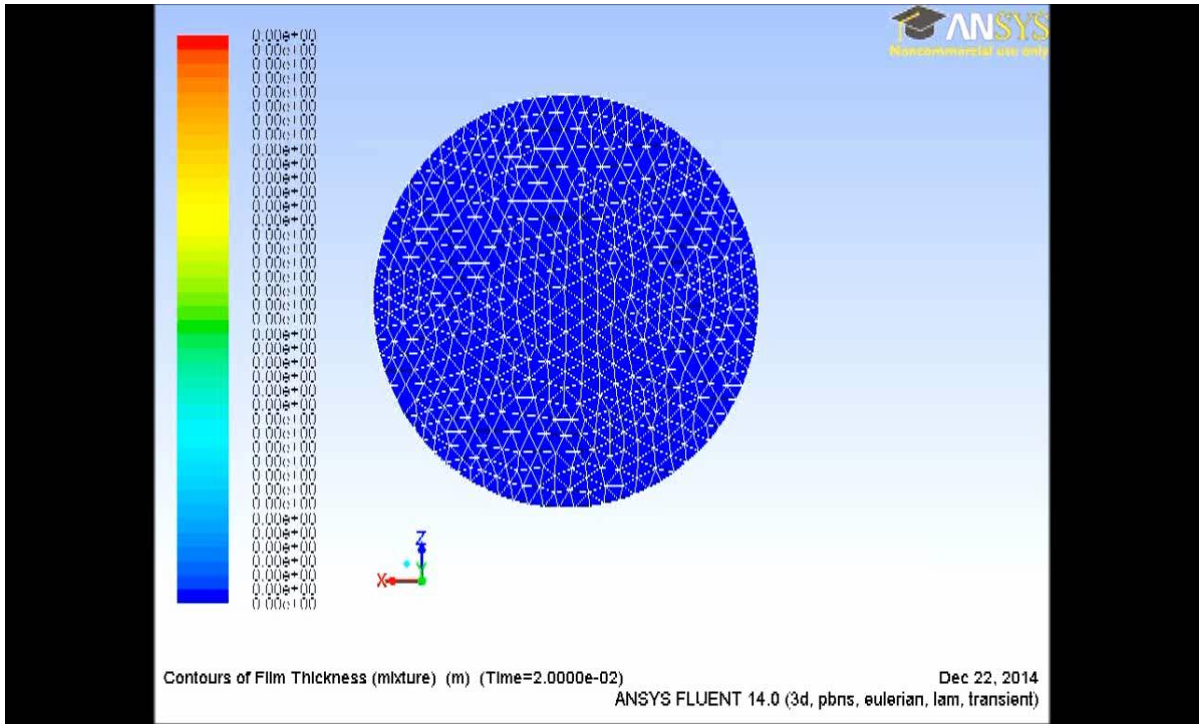
EULERIAN- EULERIAN MODEL FOR PARTICLE DEPOSITION

Multiphase model combined with **Eulerial wall film**

Air and aerosol modeled as **Eulerian fluid**

Particle deposition estimated with **fluid deposition model**



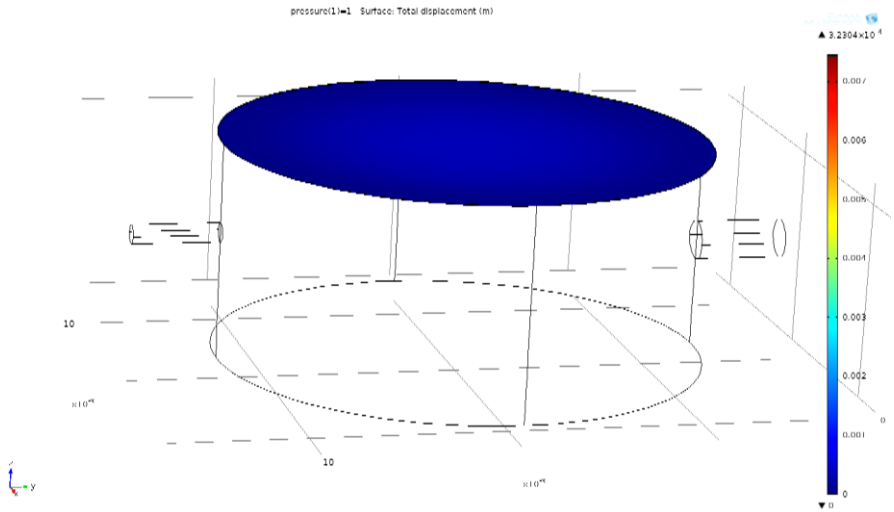


Complete deposition in 30s

Deposition efficiency of 92%

COMPUTATIONAL MODEL

- to simulate membrane displacement due to the pressure imbalance on the two sides of the air/liquid interface



[1] D. Mazzei et al., "A low shear stress modular bioreactor for connected cell culture under high flow rates"

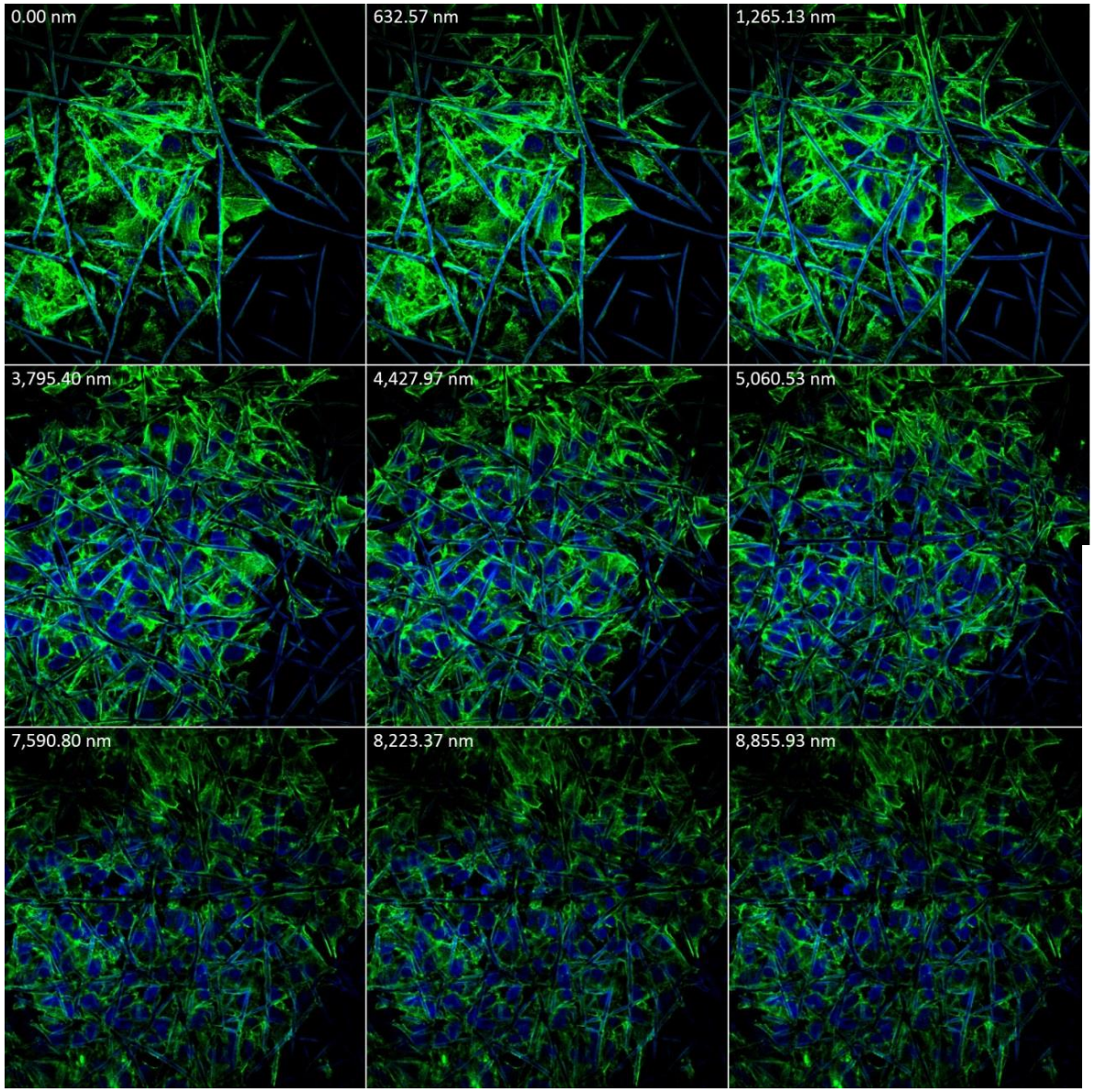
COMSOL MULTIPHYSICS®

BIOREACTOR

- Fabricated using silicone
- Apical chamber (air side) connected to an **electropneumatic regulator**
- Collector integrated to the apical chamber connects the bioreactor to the **nebulizer**
- Basolateral chamber (blood side) connected to a **hydraulic circuit**

MALI applies a max displacement of 20% with a pressure of 6kPa

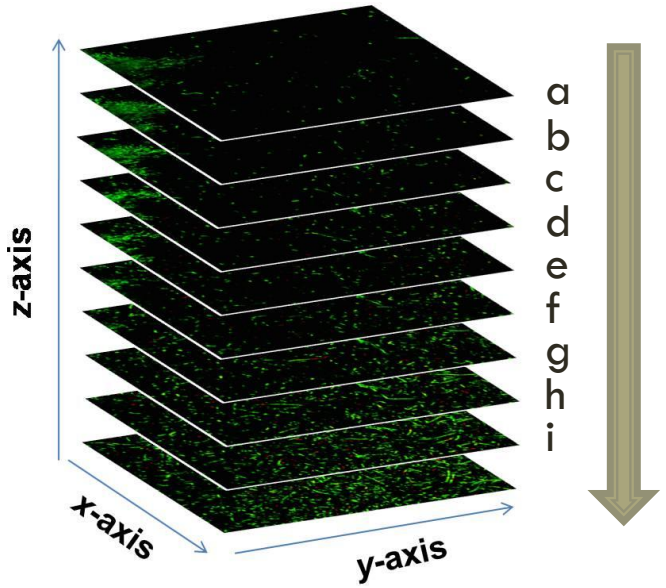
A549 Lung epithelial cells



Confocal images

Green = actin filament

Blue = cell nuclei



ADVANCED MODELS

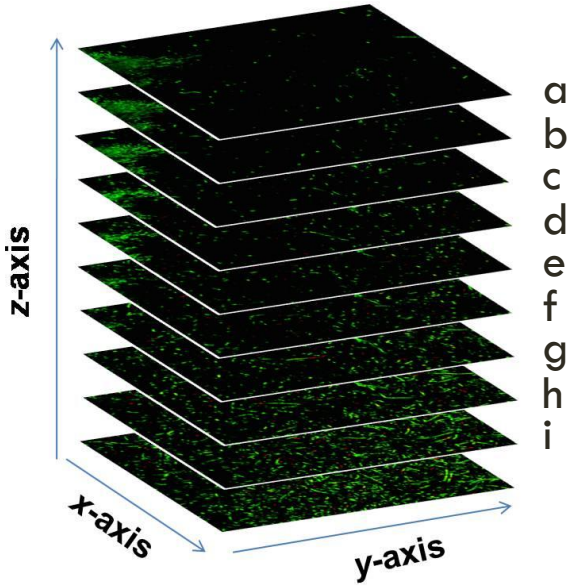
Designing of a physiologically relevant *in vitro* model of the alveolar interface according to the 3R's principle

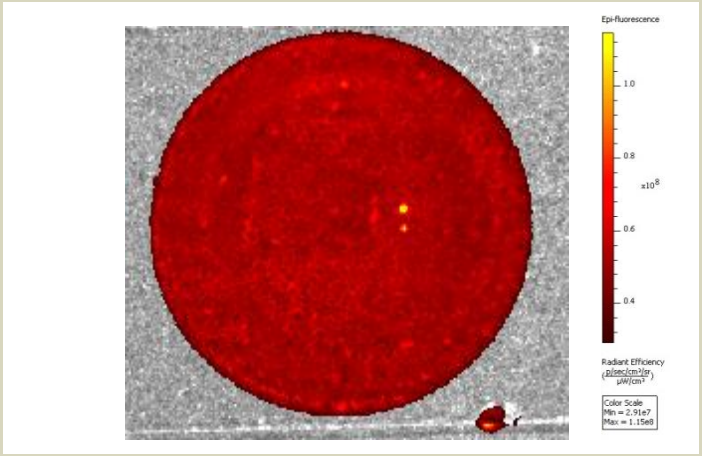
MALI system replicates the key elements of the alveolar interface

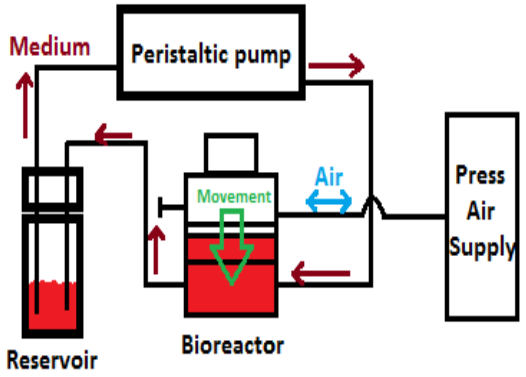
MALI can be used to model the interaction between airborne viral particles and the breathing lung

Applications in:

- Testing efficacy of vaccines
- Testing cells from patients
- Studying mechanisms of resistance and susceptibility to COVID-19





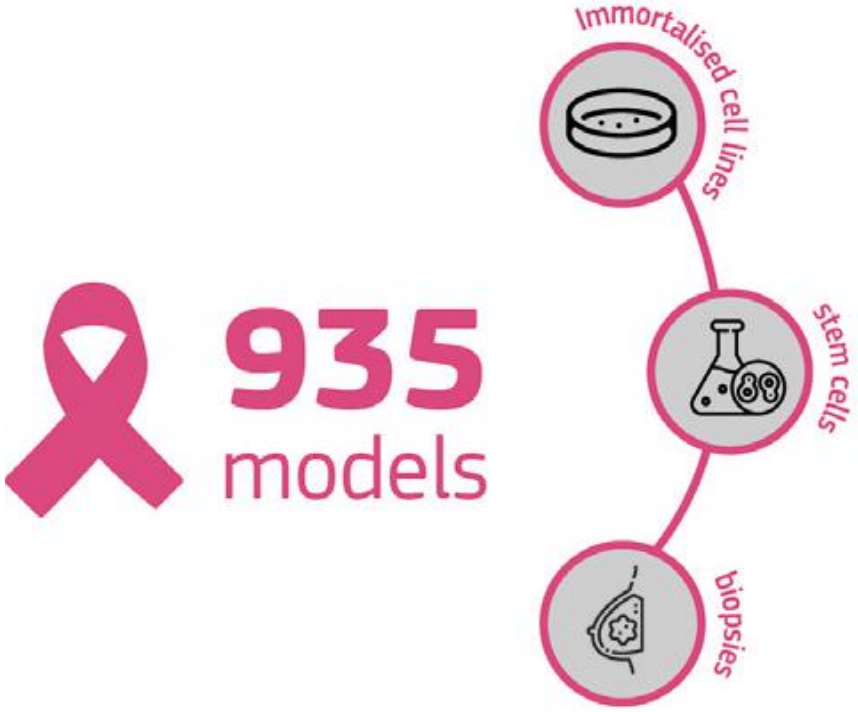


Where are we?

This review of non-animal models and methods for respiratory disease research shows that we are on the cusp of an expansion of sophisticated methods to recapitulate in vivo human lung conditions, allowing us to target specific endpoints for disease research and to greatly enhance the development of therapeutics for major lung disease, including cancer, asthma, chronic obstructive pulmonary disease (COPD) and pulmonary and cystic fibrosis.

BREAST CANCER

Folgiero, V., Romania, P., Rossi, F., Caforio, M., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Straccia, M. and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research: Breast Cancer*, EUR 30334/1 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-24689-3, doi:10.2760/618741, JRC122309.



CONCLUSIONS

The use of non-animal models is increasing each year. In parallel we also observed an increase in their usage for drug development and testing, which indicates the importance of finding new and more effective treatments for breast cancer. On the other hand, the publication of new human-based models for breast cancer research was steady. However, the use of 3D models has increased suggesting a better approximation to breast cancer physiology, especially through the use of spheroids.

The main disease features addressed were breast cancer initiation and development at cellular levels by using mainly immortalised cell lines in particular, and *ex vivo* models such as tumour biopsies. The main interest in breast cancer initiation and development among the research community mirror the great importance in discovering the molecular bases of the starting events.

Human breast cancer immortalised cells have been employed in 747 peer reviewed publications to study breast cancer initiation, treatments, metastatic process and the microenvironment-tumour interactions using several culturing conditions.

Cosa sono le 3R?

Replacement, Reduction, Refinement

Responsible, Rigorous, Reproducible

Ricerca Responsabile=Ricerca di Qualita'

Martedi 2 febbraio 2021 ore 15.00-18.00
Registrazioni: centro3r.it/eventi

3 anni, 3R: didattica, ricerca e le prossime sfide

Un evento online rivolto al pubblico e agli studenti, docenti e ricercatori.

Didattica:

- *Dove posso imparare la teoria e pratica delle 3R? Quali corsi universitari?;*

Ricerca:

- *Sinergia tra ingegneri e biologi per lo sviluppo di modelli tecnologici funzionali;*
- *Avvicinarsi alle 3R con tecnologie di Intelligenza Artificiale*

La sfida:

- *Integrazione tra la sperimentazione animale e le 3R per una ricerca di qualità;*

Registrare qui: https://www.centro3r.it/it/events/3-anni-3r-didattica-ricerca-e-prossime-sfide

Centro 3R

L'era delle 3R: modelli in silico, in vitro e in vivo per promuovere la ricerca traslazionale

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