LUNG & PARTICLE RESEARCH GROUP

THE NHBE AND METABO-LUNG™ MODELS: NORMAL AND METABOLISING *IN VITRO* ALTERNATIVES FOR LUNG RESEARCH

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PRIFYSGOL CAERDYDD

The Burden of Lung Disease

GLOBAL BURDEN OF LUNG DISEASE

- Globally respiratory disease ranks 2nd; In the UK & Western Europe it's ranked 1st[1]
- The FDA stated that no significant advances have been made in diagnostic tools or drug treatments for lung disease in the last 25 years[2]
- Outdoor air pollution has increased globally[2]
- WHO predict a 30 % increase in chronic respiratory diseases in the next 10 years[3]
- By 2020, lung disease will cause 11.9 million deaths worldwide[1]

[1] European Lung Foundation [2] BéruBé (2011), ATLA [3] WHO

COST OF LUNG DISEASE

- **In UK respiratory illness cost £6.6 billion[1]:**

  £3.0 billion in NHS care

  £1.9 billion in mortality

  £1.7 billion in morbidity

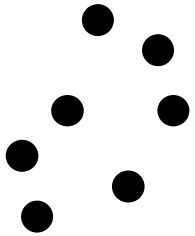
- **Lung disease in Europe costs nearly €102 billion[2]**

- **In the US respiratory diseases cost almost $15 billion in 2010[3]**

[1] British Lung Foundation [2] European Lung Foundation [3] BéruBé (2011), ATLA

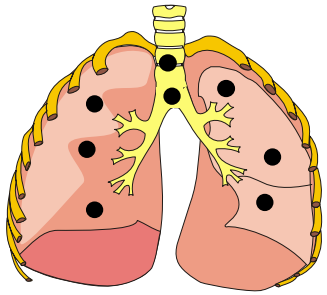
Traditional Testing: Rodent Lung

Rodent



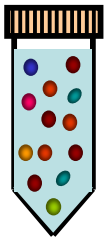
Instil/inhale Particles

Acute/Chronic Exposure



Euthanise Rodents

Lavage & Embedded Tissue



Cells/Proteins & Microscopy

Identify Biomarkers

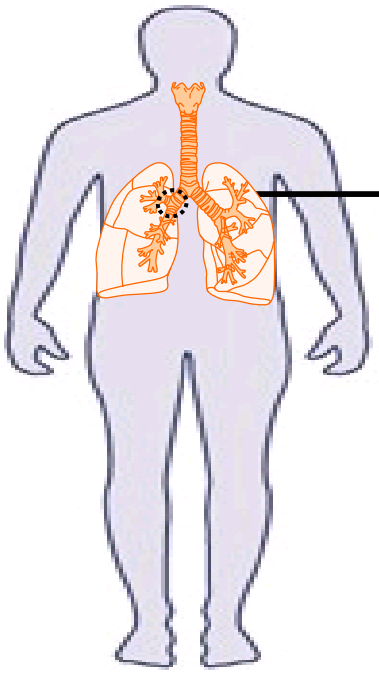
Extrapolate

Extrapolated Human Lung Responses

BéruBé et al. (2009), ATLA

IN VITRO LUNG MODELS

MICRO-LUNG™

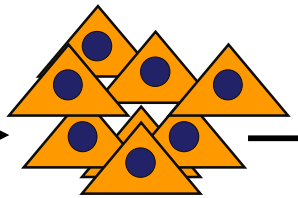


Normal Human Bronchial Epithelium

(NHBE)

Isolation

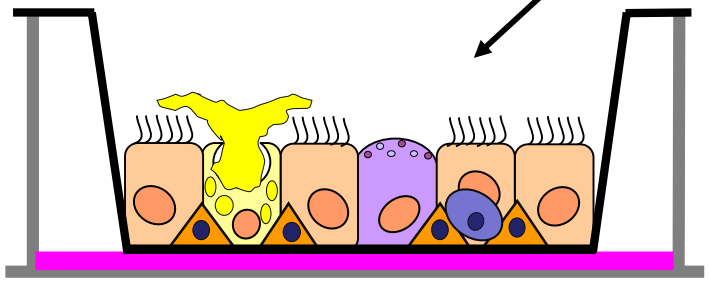
BE Cells



Basal Cells (Progenitor)

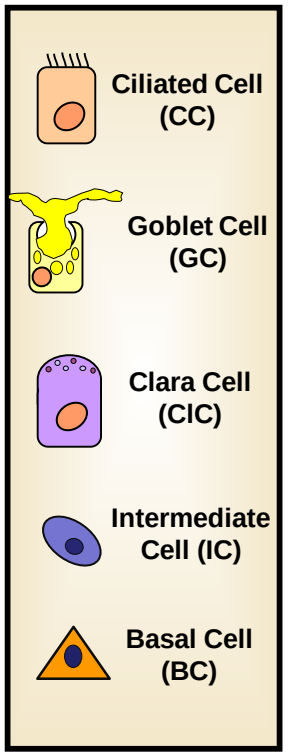
Proliferation (Progenitor)

Differentiation



Micro-Lung™ MODEL

Millipore® Insert (Microporous Membrane)



Toxicological Analysis

Morphological Analysis

Inter-Omic Analysis

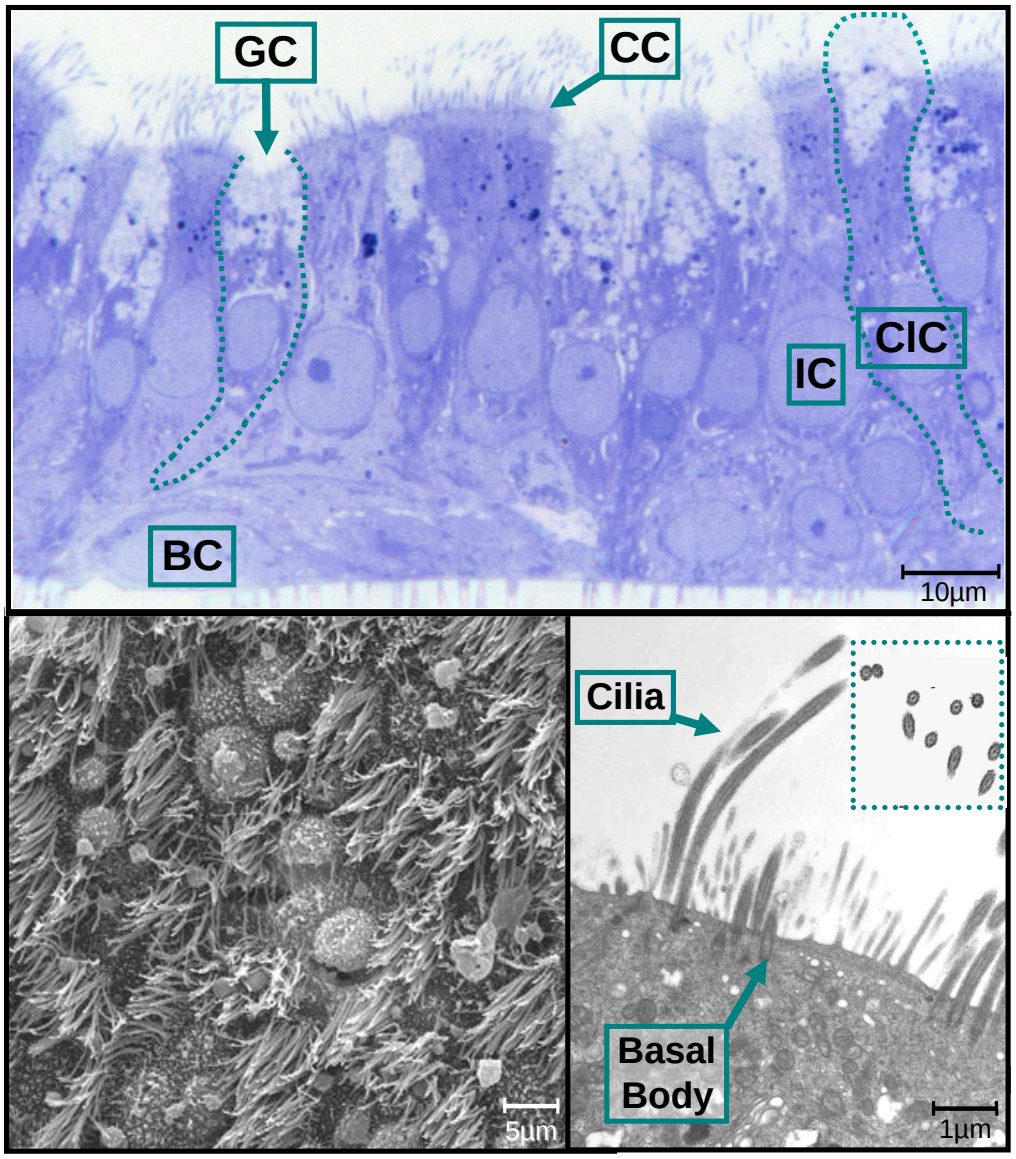
Prytherch & BéruBé (2014), Pan Stanford Publishing

Prytherch & BéruBé (2015), RSC Drug Dis

WHY THE NHBE MODEL?

- **PSEUDO-STRATIFIED, DIFFERENTIATED CULTURES**
  - Ciliated, goblet, Clara, serous, intermediate and basal cells in multiple layers
- **FORM TIGHT & ADHEREN JUNCTION**
- **FORM DESMOSOMES**
- **FORM CILIA & MICROVILLI**
- **EXPRESS CYTOKINES & SECRETE MUCIN**
- **NO CANCEROUS OR TRANSFECTED GENES**

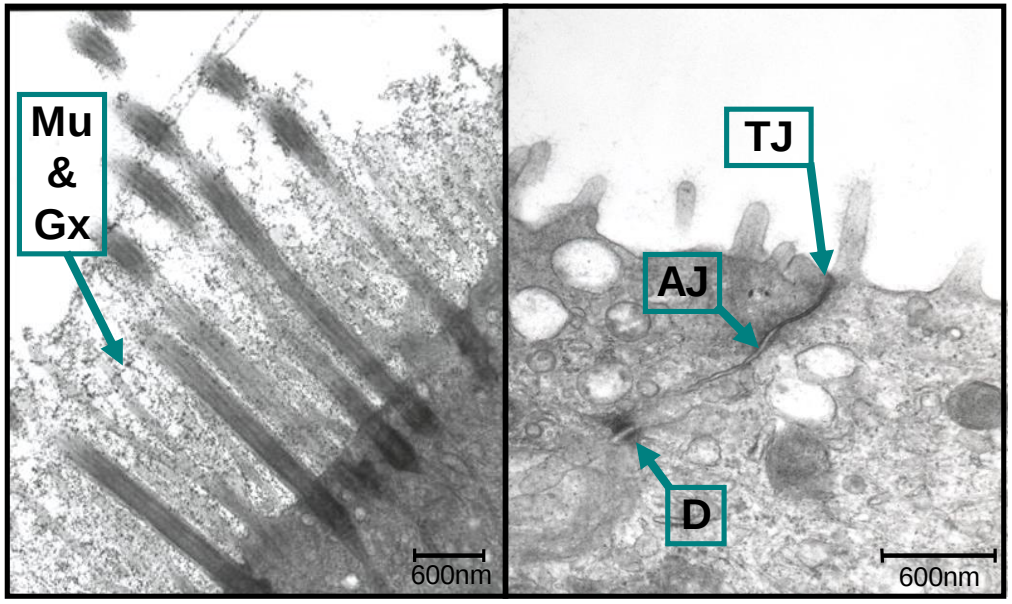
MUCO-CILIARY PHENOTYPE

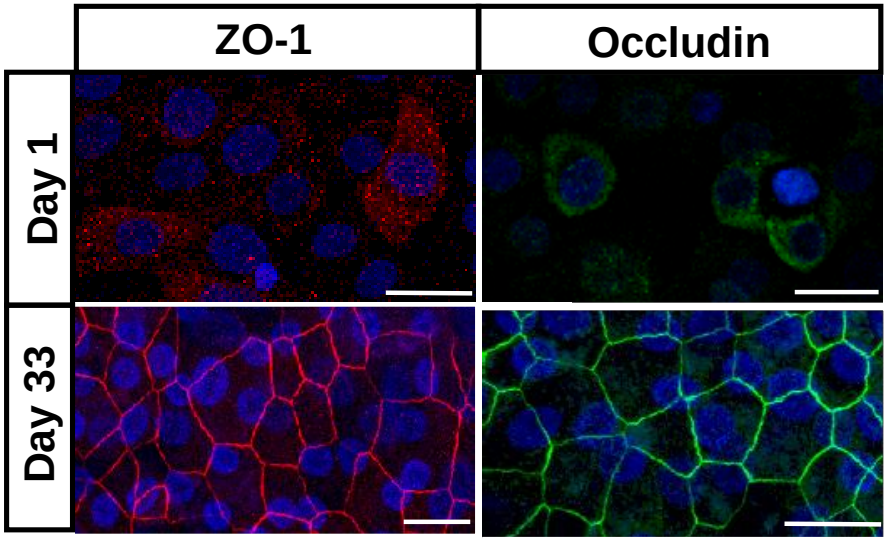


- GC = Goblet Cell
- CC = Ciliated Cell
- ClC = Clara Cell
- IC = Intermediate Cell
- BC = Basal Cell

Prytherch et al. (2011), Mac Biosci

SECRETIONS & JUNCTIONS



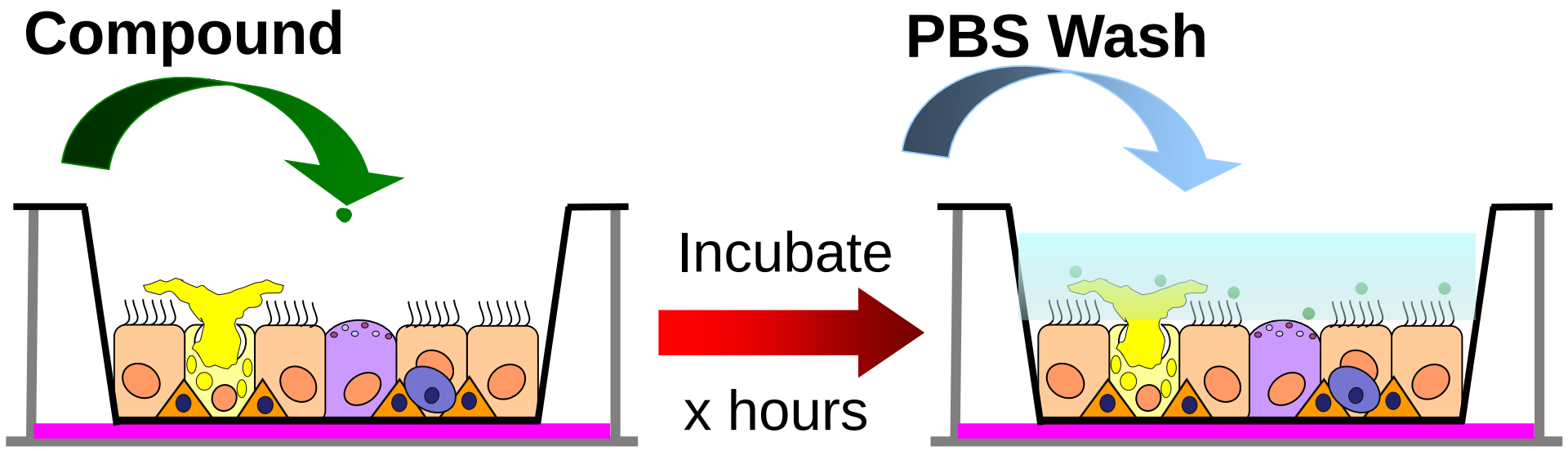


- Mu = Mucin
- Gx = Glycocalyx
- TJ = Tight Junction
- AJ = Adherens Junction
- D = Desomsome

Prytherch et al. (2011), Mac Biosci

TOXICOLOGICAL TEST SYSTEM

TOXICOLOGICAL TEST SYSTEM



Characterisation

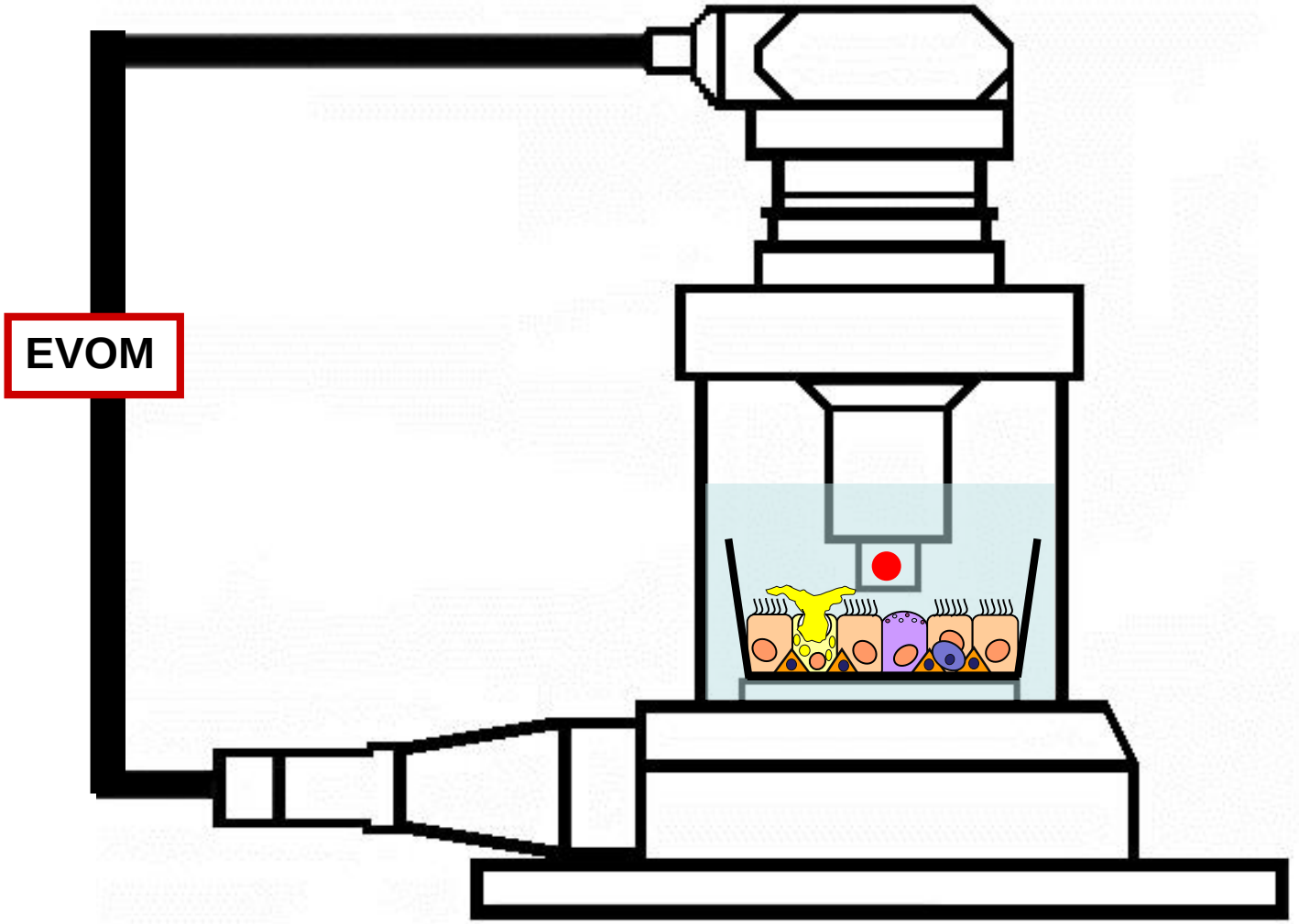
Toxicological Analysis

Biomarkers

BéruBé et al. (2009), ATLA

TRANS-EPITHELIAL ELECTRICAL RESISTANCE

- Endohm Chamber

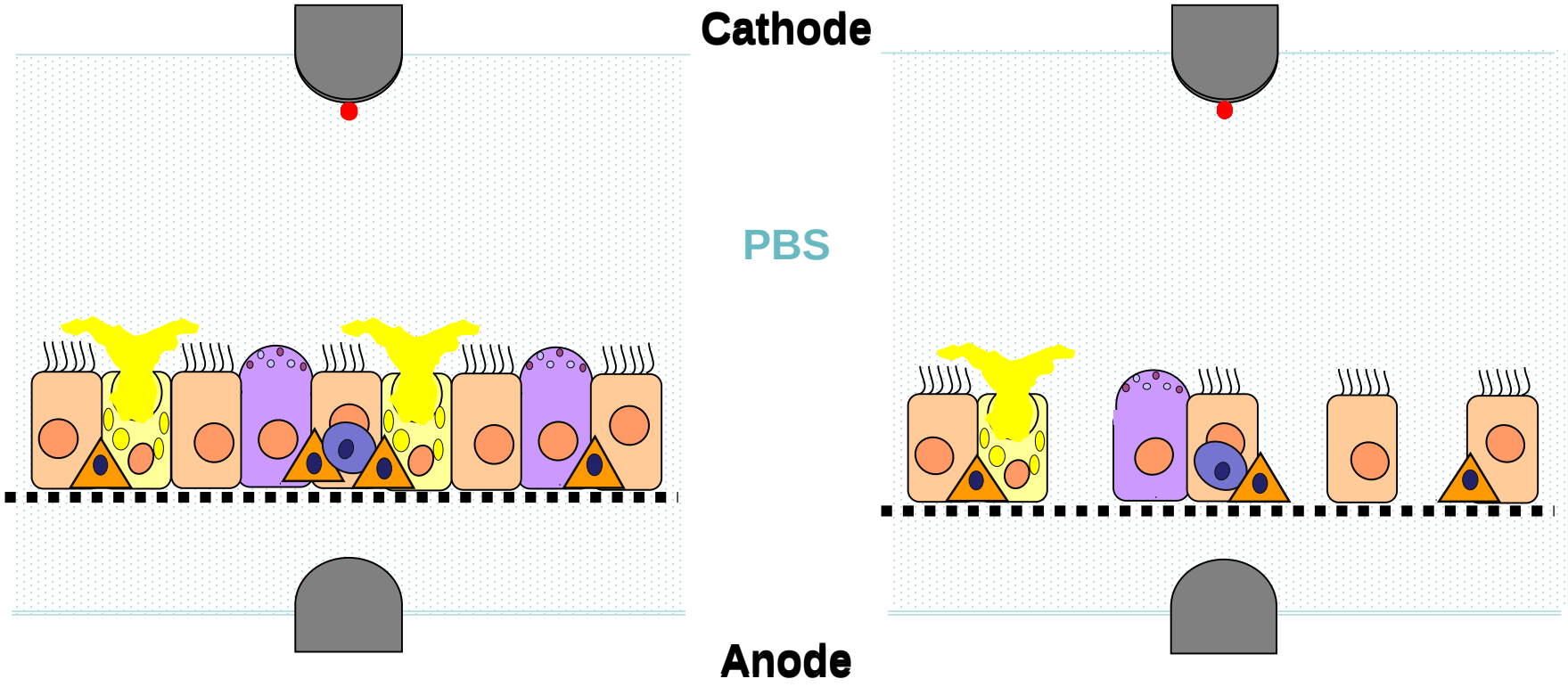


BéruBé et al. (2009), ATLA

TEER

High Resistance

Low Resistance

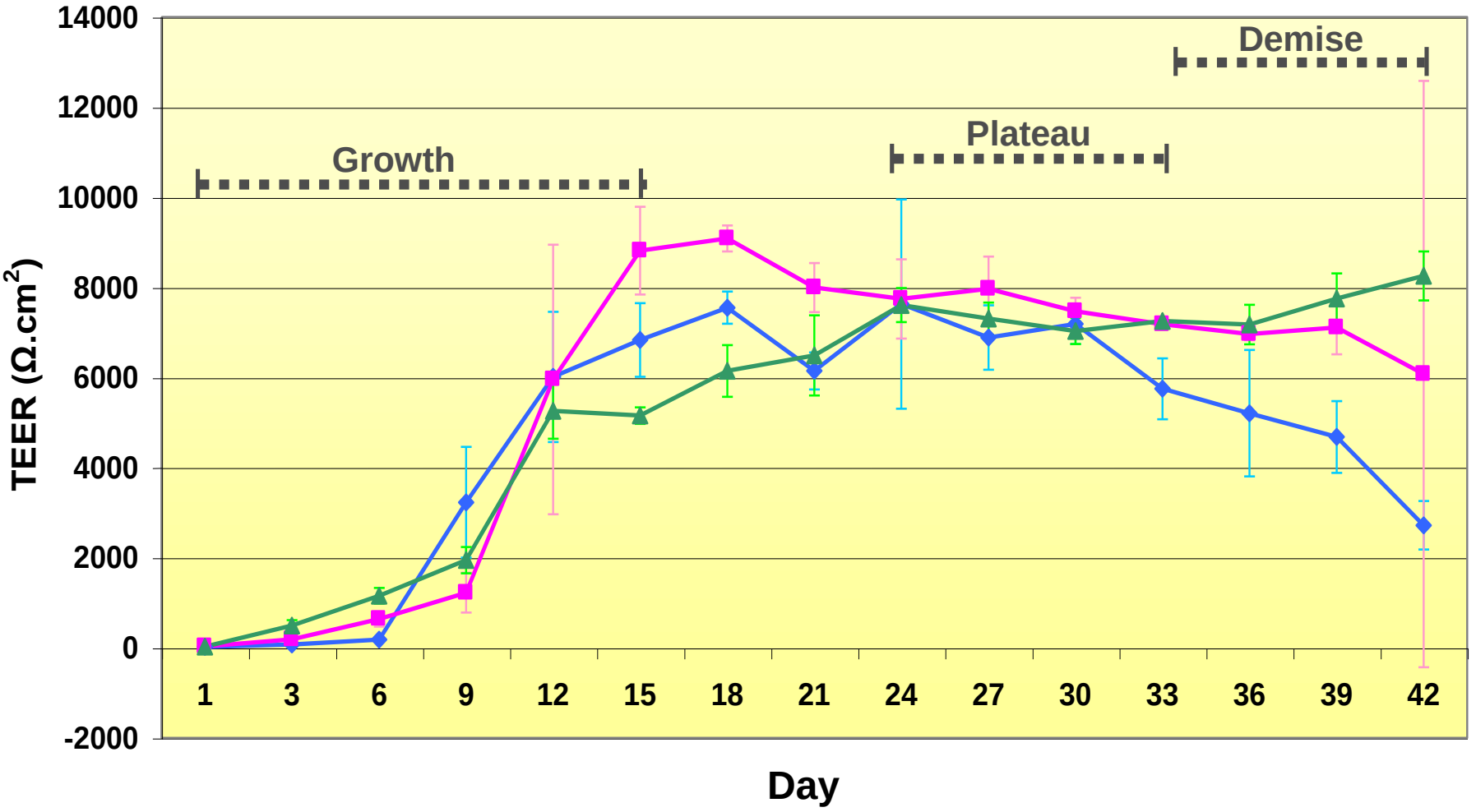


~ 2000 Ω

~ 300 Ω

BéruBé et al. (2009), ATLA

TRANS-EPITHELIAL ELECTRICAL RESISTANCE



TEER values denoting minimal variability between donors (*n*=3) during growth, plateau and demise; Error = Std. Dev

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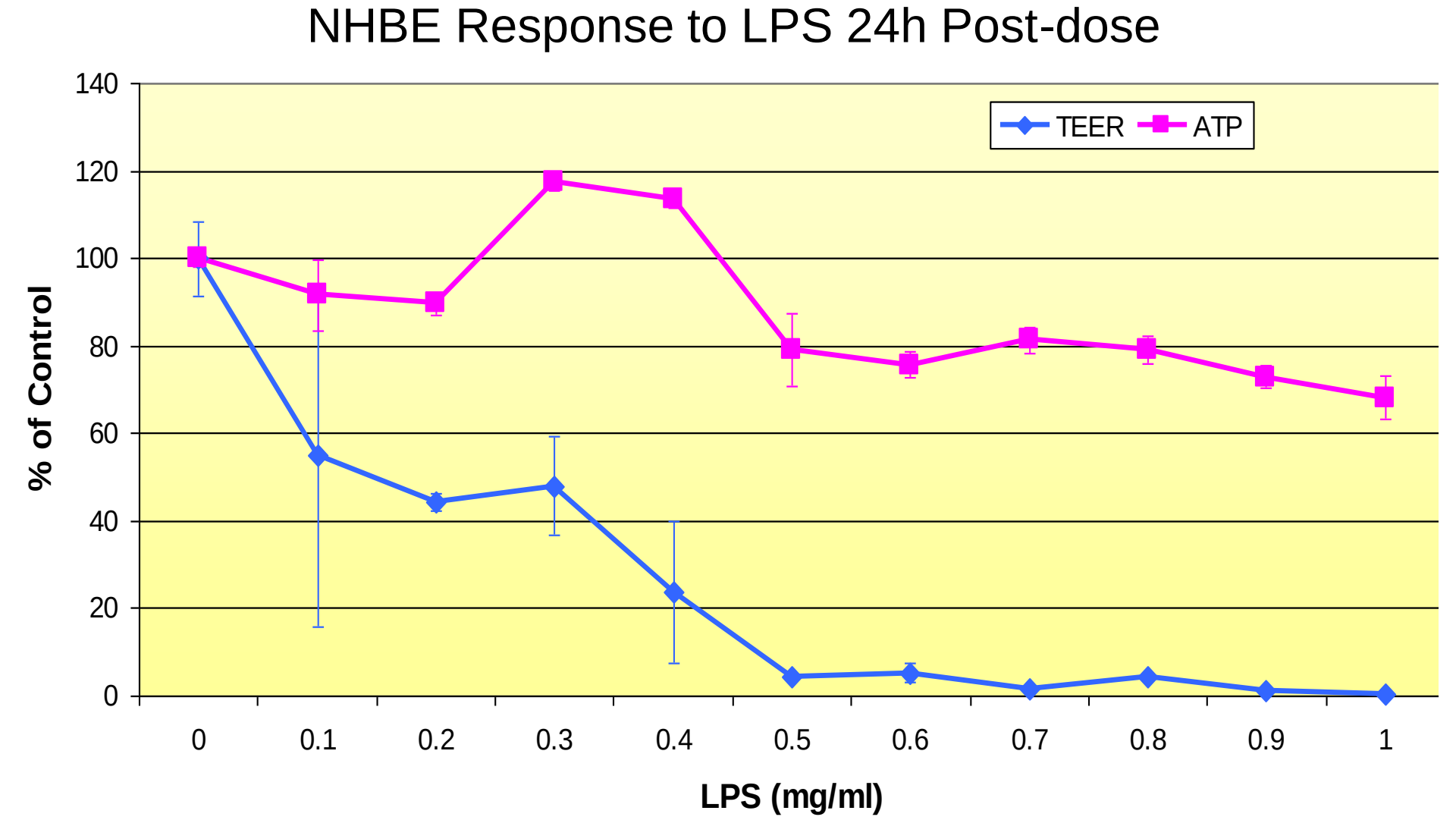
LPS TOXICOLOGY

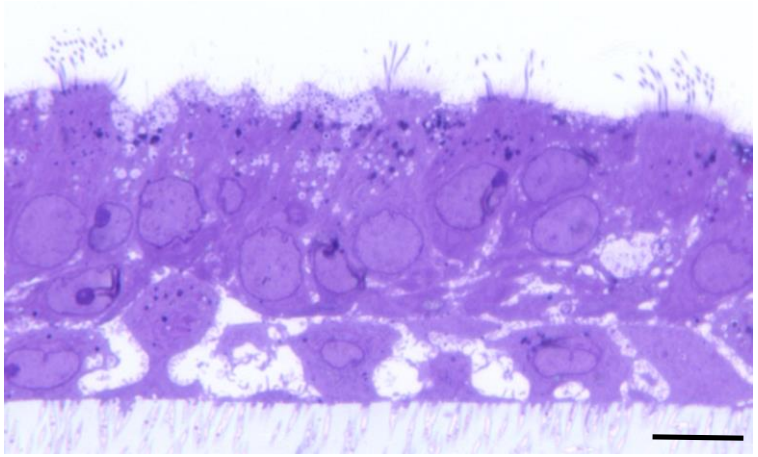
LPS - TOXICOLOGY

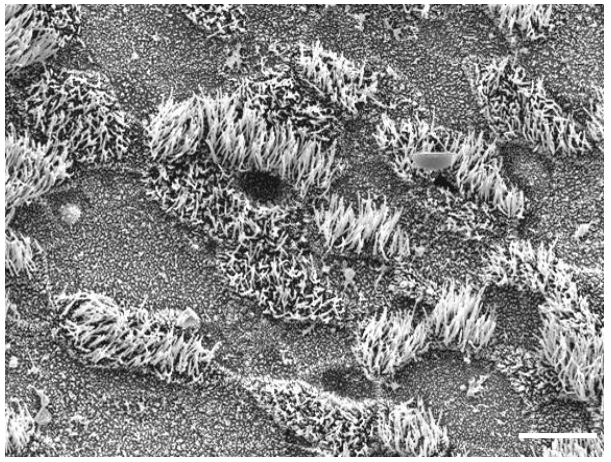


Prytherch (2010) PhD CU

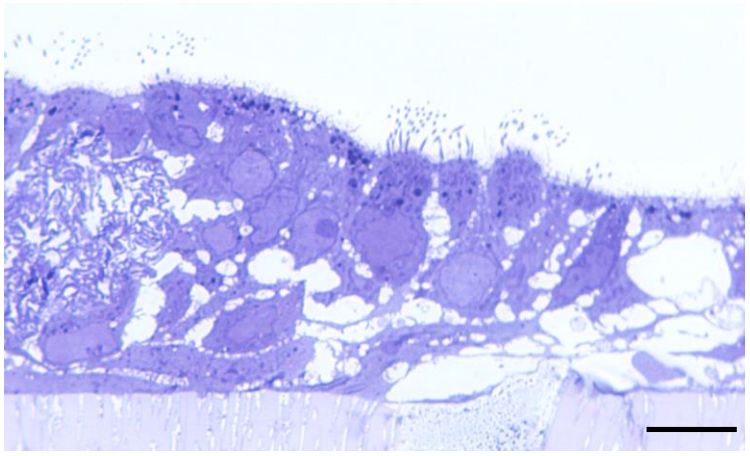
LPS - TOXICOLOGY

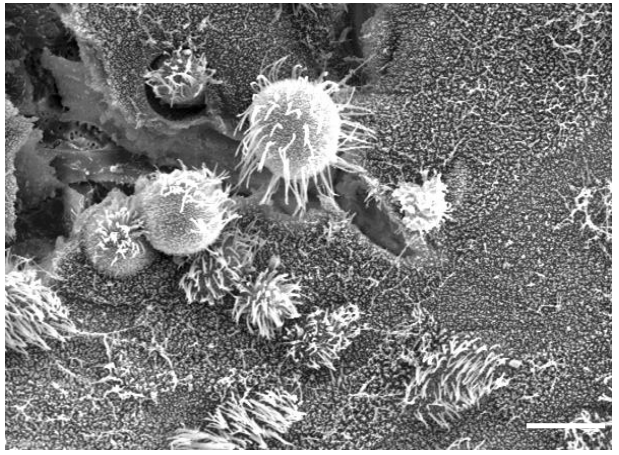
LPS: TD_5 (0.1mg/ml)





LPS: TD_{20} (0.5mg/ml)





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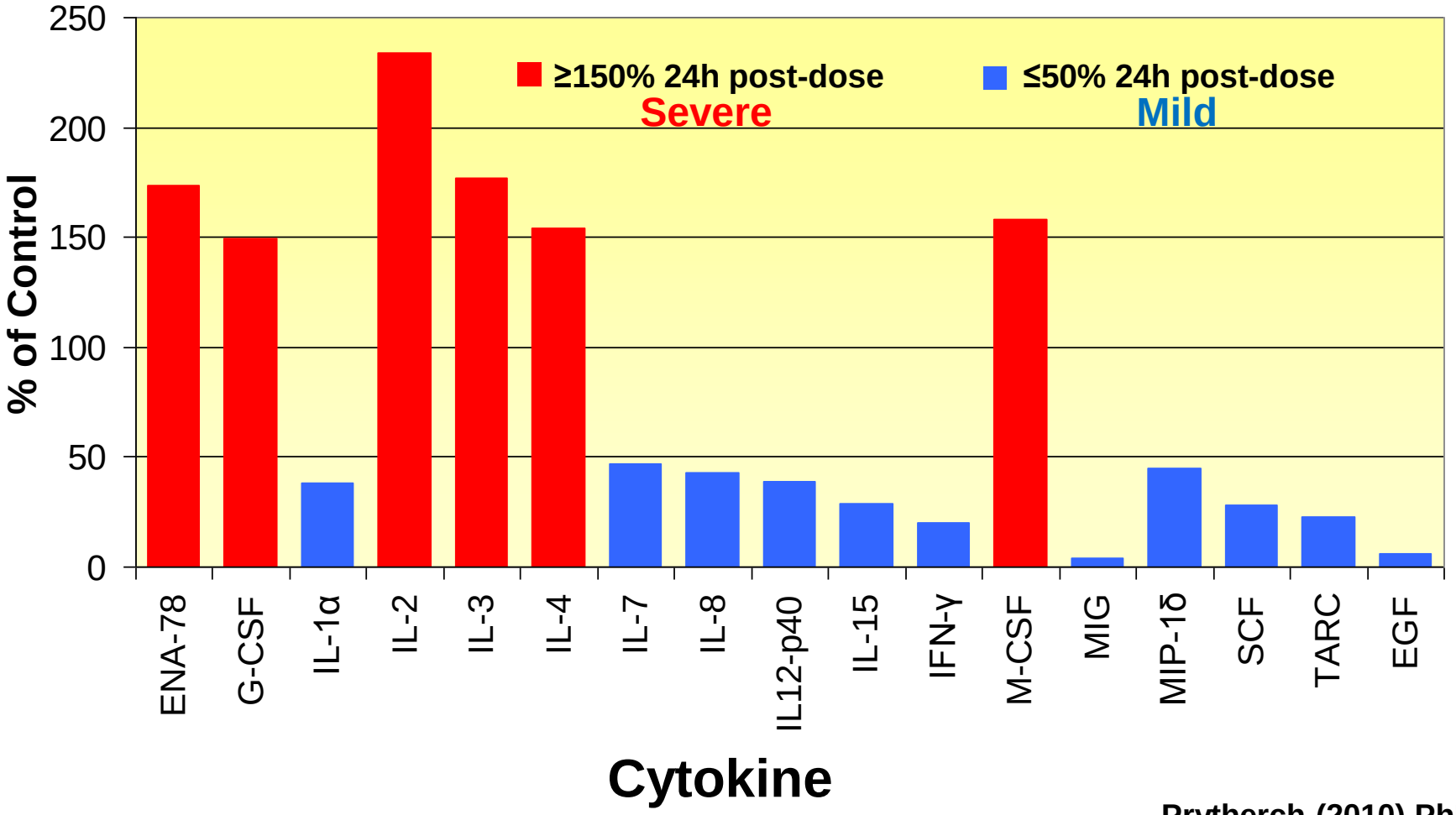
LPS - TOXICOLOGY

Major Cytokine Responses to LPS (TD_5: 0.1mg/ml)



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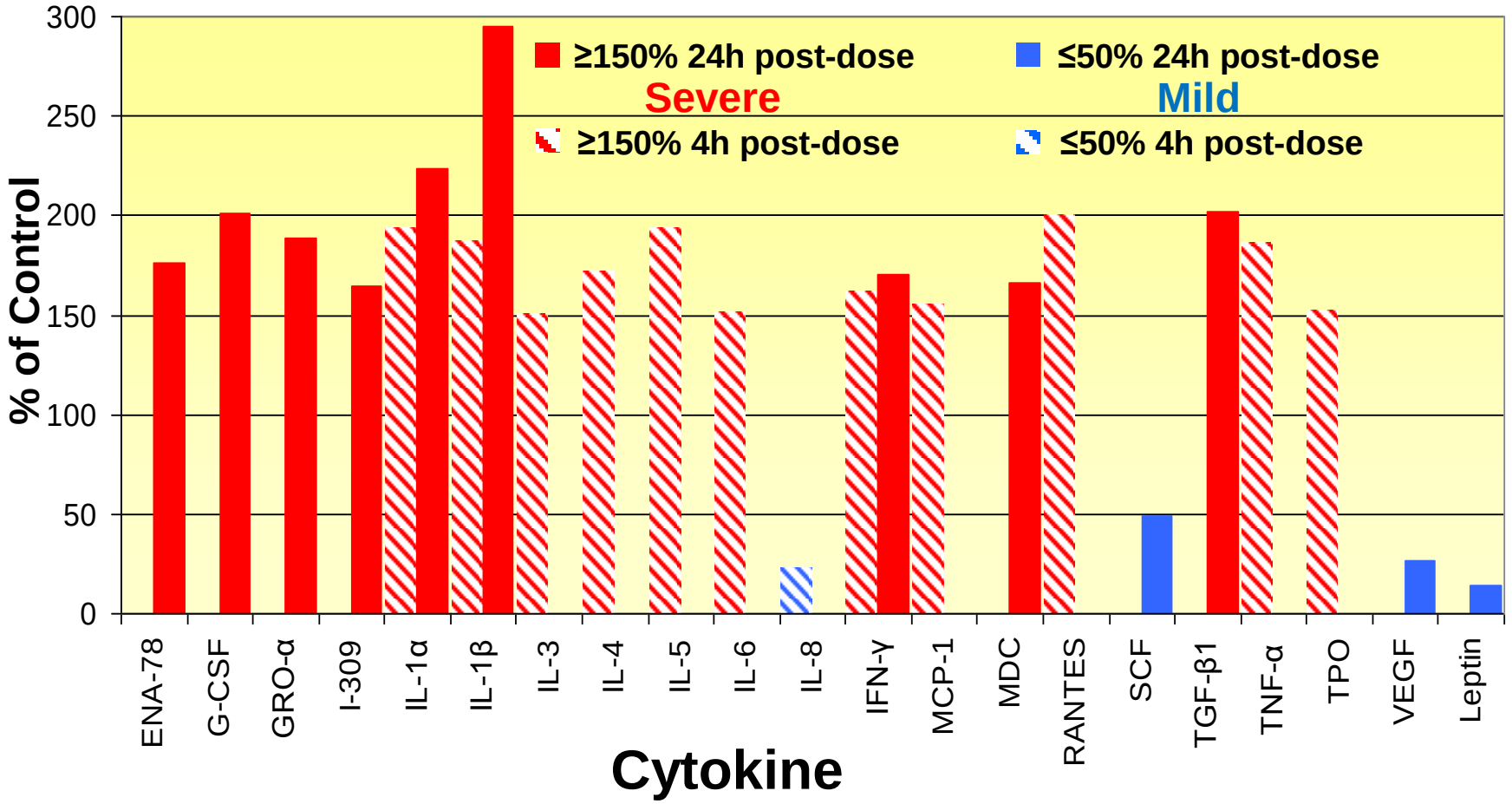
LPS - TOXICOLOGY

Major Cytokine Responses to LPS (TD_{20}: 0.5mg/ml)



Prytherch (2010) PhD CU

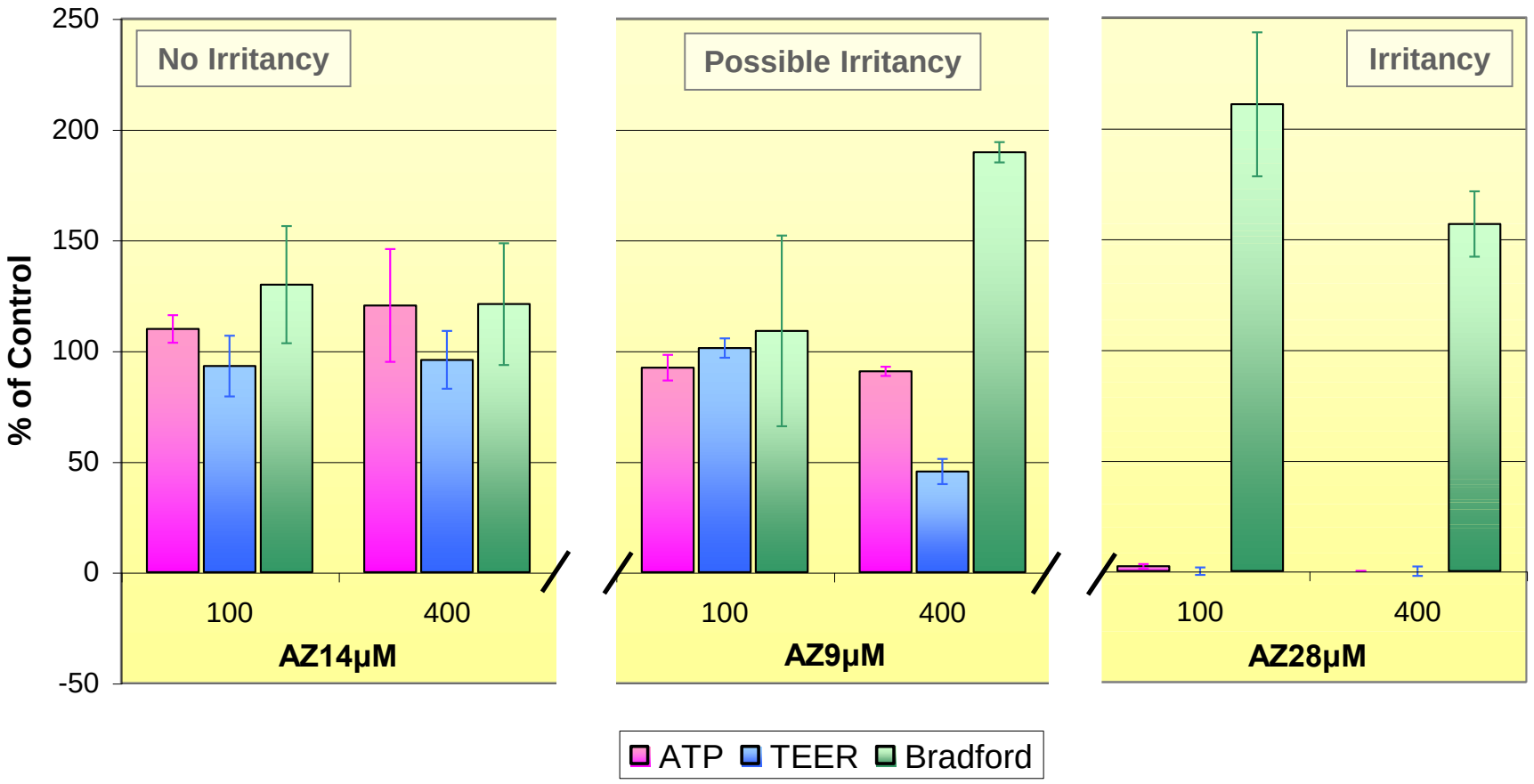
LPS - TOXICOLOGY

Correlations between Cytokines Changes post-LPS Treatment

		TD_5-24h	TD_{20}- 4h	TD_{20}-24h
Mediators of injury	1	ENA-78	---	ENA-78
		G-CSF	---	G-CSF
		SCF	---	SCF
Discerning (low to severe) injury	2	IL-1 α	IL-1 α	IL-1 α
		IFN-γ	IFN-γ	IFN-γ
Severe injury	3	---	IL-1β	IL-1β
Early indicators of injury	4	---	IL-5	---
		---	IL-6	---
		---	RANTES	---
		---	TPO	---

IN VIVO COMPARISON

BIOCHEMICAL IRRITANCY



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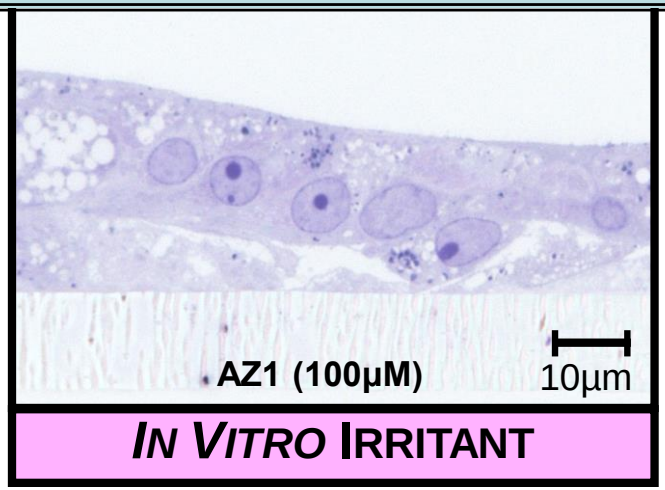
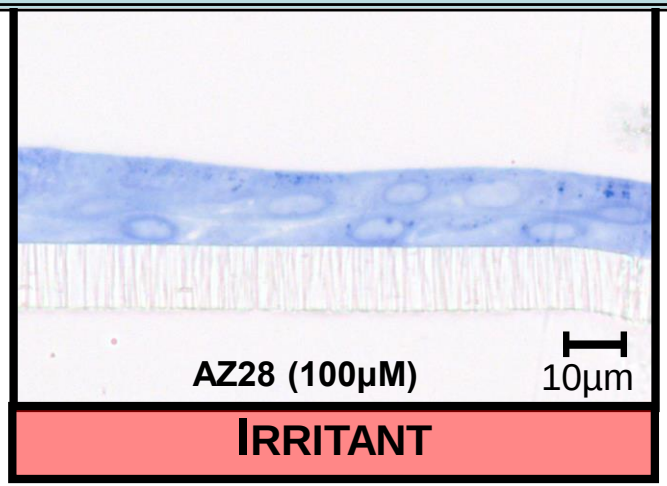
MORPHOLOGICAL IRRITANCY

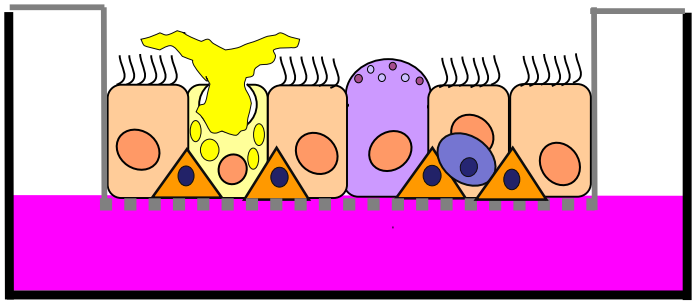
- **Correlation between the NHBE *in vitro* model and the rat *in vivo* model was 76.9%**





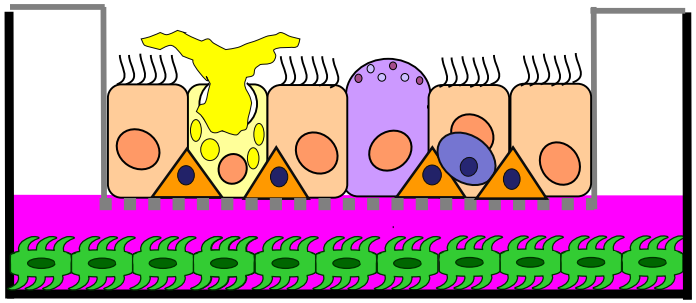
BUILDING METABOLISM

METABO-LUNG™



NHBE MODEL

HEPATOCYTES



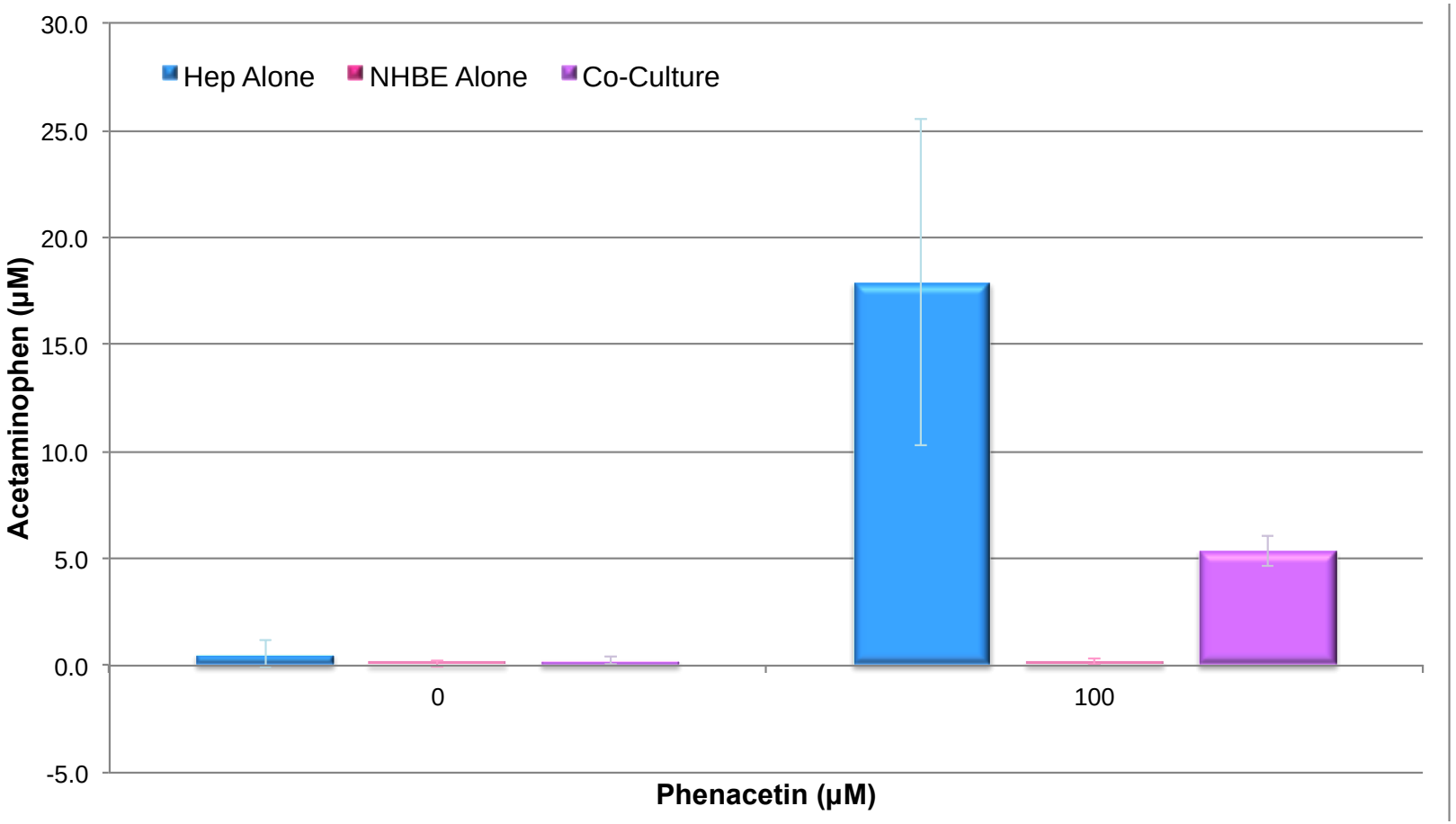
METABO-LUNG™

Patent:
WO2012/123712

BéruBé (2011) Biotech Inter

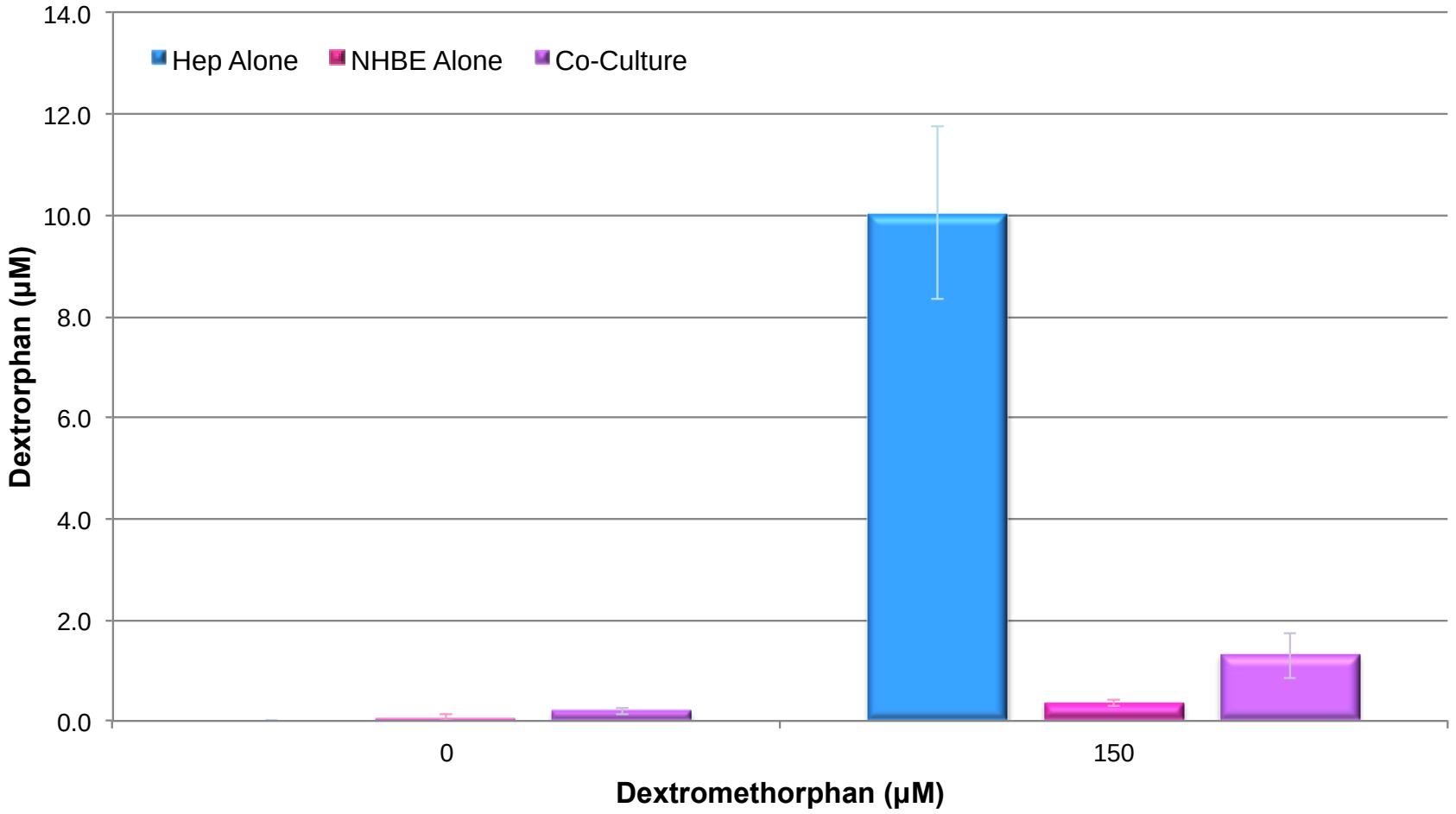
BéruBé (2011) Advances Wales

HUMAN CYP1A1 ACTIVITY



Detection of Acetaminophen: 24hrs post-incubation with Phenacetin; Error = Std. Dev

HUMAN CYP2D6 ACTIVITY



Detection of Dextrorphan: 24hrs post-incubation with Dextromethorphan; Error = Std. Dev

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CONCLUSIONS

CONCLUSION 1: LUNG DISEASE

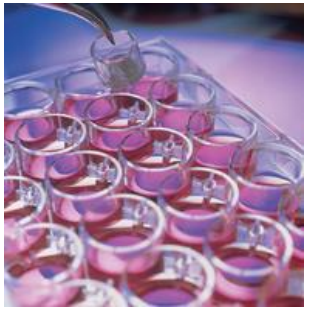
- Human lung disease is a major cause of **morbidity**, **mortality** and **economic burden**
- Cutting-edge research **centers** on understanding lung injury/repair; **patho-physiology models urgently needed**
- Emerging therapies may have **greater success** if developed by route of **human tissue-based model systems**

CONCLUSION 2: NHBE MODEL

- Extensive **characterisation** of the model during normal growth and toxic challenge, suggest it is a **viable, *in vitro*, test system** of the **respiratory epithelium**

- **Exposure window**: acute, chronic, repeat

- **Cost-effective**: 1 donor cryovial (Lonza) yields **350** inserts (Millicell®, 5mm dia.)



CONCLUSION 3: REPLACEMENT

- **Metabo-Lung™** - Integration of lung and metabolic component: **multi-organ toxicity**
- **Filter-well bioreactors** support assembly of complex tissues within an affordable **high-throughput** framework
- Suitable for practical applications at **all research levels** & create an **immediate impact on the 3Rs (i.e. 'Replacement')**

AKNOWLEDGEMENTS & THANK YOU!

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LUNG and PARTICLES RESEARCH GROUP

Institute For Science And Health

THE 2013 LUSH PRIZE
SUPPORTING ANIMAL-FREE TESTING

Unilever

DETR
Department of the Environment, Transport and the Regions

The Nuffield Foundation

AstraZeneca
UNITED KINGDOM

WDA

Llywodraeth Cynulliad Cymru
Welsh Assembly Government

abpi

bbsrc
biotechnology and biological sciences research council

FRAME

Medical Research Council MRC

NC3Rs

wellcome trust

Natural Environment Research Council

Putting patient safety first
Safer Medicines Campaign

THE ROYAL SOCIETY