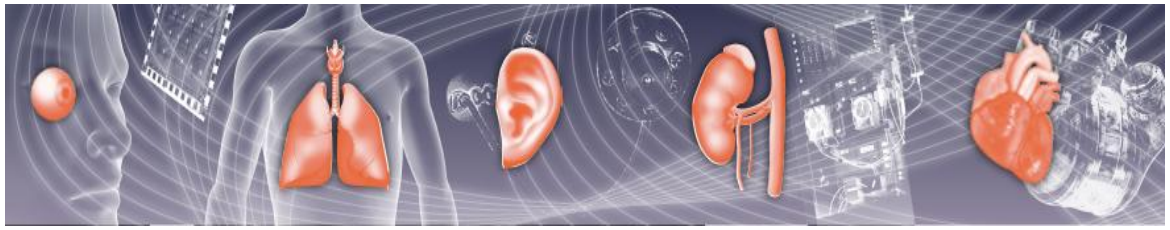

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Advanced microfluidic-based in vitro models for lung research

Prof. Dr. Olivier Guenat

University of Bern, Medical Faculty,
ARTORG Center, Lung Regeneration Technologies Lab
Switzerland

EUSAAT 2012 Conference, Linz, Austria

ARTORG Biomedical Engineering Center

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ARTORG: Artifical Organs Center

10 laboratories closely collaborating with specific clinics from the University Hospital of Bern

INSELSPITAL
UNIVERSITÄTSSPITAL BERN
HOPITAL UNIVERSITAIRE DE BERNE
BERN UNIVERSITY HOSPITAL

ARTORG Lung Regeneration Technology Group (created Nov. 2009)

- Pulmonary Medicine Division & Thoracic Surgery Clinic
- Develop novel in-vitro models that better reproduce in-vivo like lung conditions (perfusion, respiratory movements, 3D microenvironment, ...)
- **Combining Engineering, Microtechnology and Cell Biology**

High attrition rate in drug discovery

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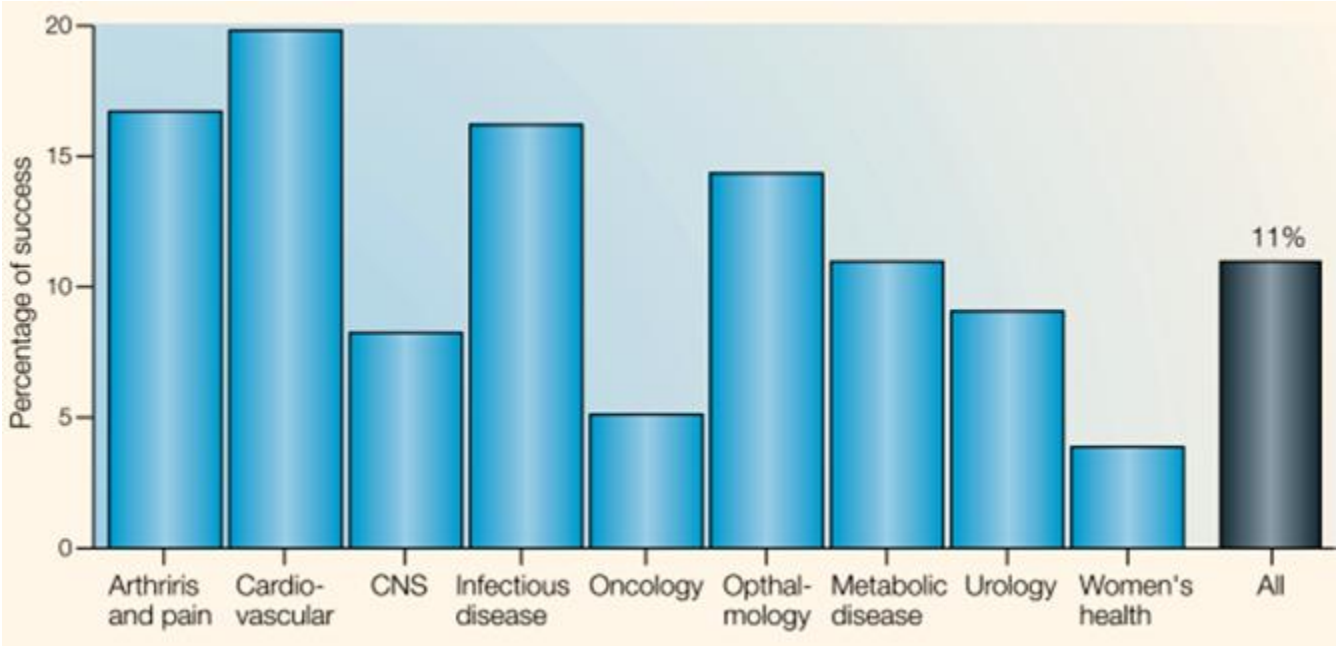

1991-2000: 10 biggest pharma companies

Kola, Nature Review, 2004

11% overall success rate (large part of the failure happens during clinical trials)

Mainly due to: toxicology, safety and efficacy issues

=> Lead to important costs increase (clinical trials only: 100-800mio $/drug)

FDA approved respiratory drugs (1995-2012)

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- No approved treatment for many respiratory diseases

⇒ Need for more predictive assays prior to clinical trials
⇒ Assays that better reproduce the human in-vivo situation of the lungs

Towards in-vitro models of pulmonary diseases

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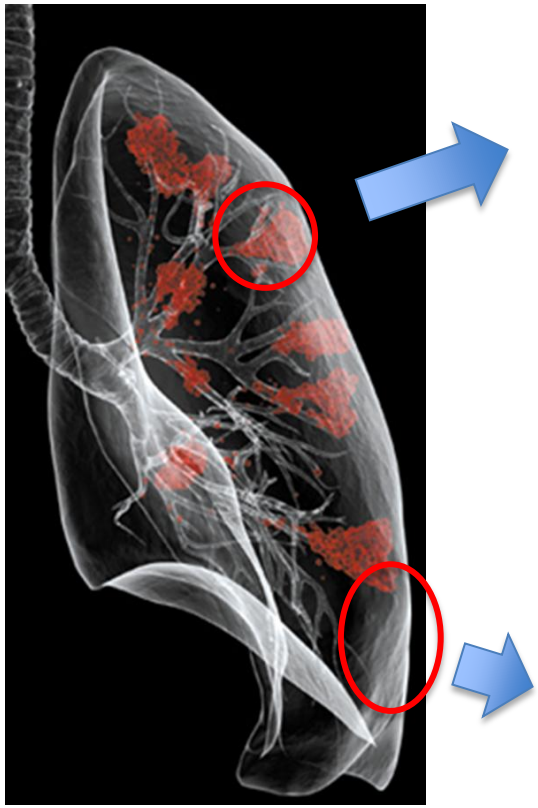

Pulmonary Fibrosis:

Median survival 3yrs
> 45'000 cases/year (US)
Prevalence (+150% since 2001)

No effective treatment

Repeated microinjuries of lung epithelial layer with abnormal wound repair

Malignant Pleural Mesothelioma:

rare disease: 160 cases/year (CH)
median survival: +/- 12 months

No effective treatment

60-70% cases due to asbestos
Main problem: disease recurrence

Wound-healing assay on chip

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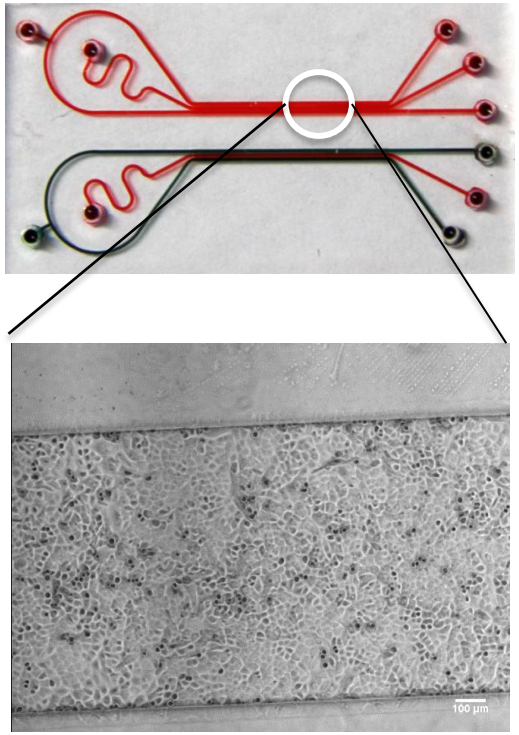
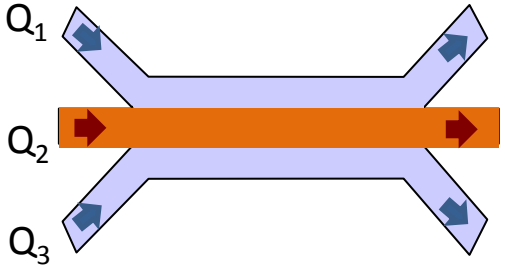
Mimic epithelial microinjury in a microfabricated channel

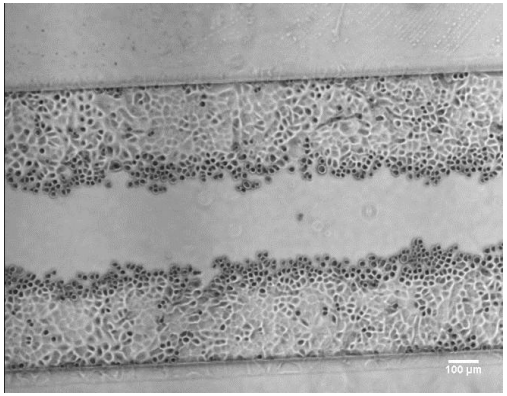
Confluent epithelial layer



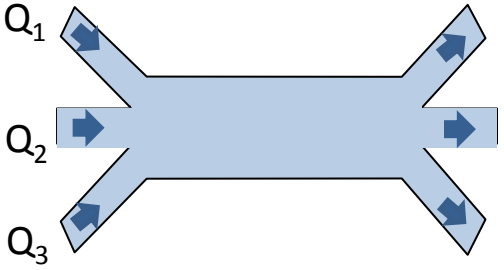
Creation of a microinjury
Focused flow of trypsin

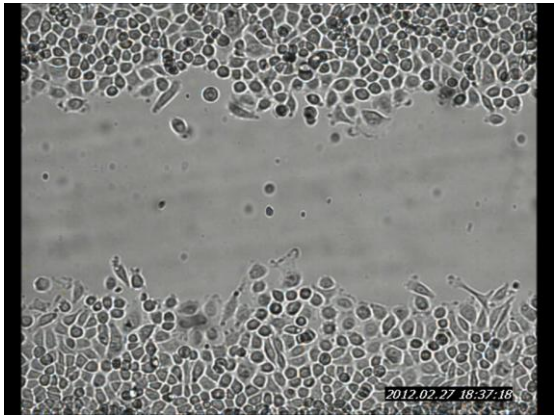


100-300um wide wounds



Wound-healing
Perfused flow





Perfused wound healing

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- **HGF (hepatic growth factor):** potent mitogenic factor known to promote the proliferation of lung epithelial cells (Gazdhar et al, AJP Lung Phys., 2007)

  Method: perfusion of 10ng/ml HGF exposed during healing

- **Mesenchymal stem cells** are known to reduce bleomycin-induced lung injury, possibly by promoting epithelial proliferation (Aguilar et al, PlosOne, 2009)

  Method: the supernatant of a rat BMSC culture was perfused on the epithelial layer

Questions: a) does reepithelialisation occur in a microfluidic system?
b) is the phenotype of the cells preserved?
c) differences between in-vitro and microfluidic models?

Wound-healing in a microfluidic channel

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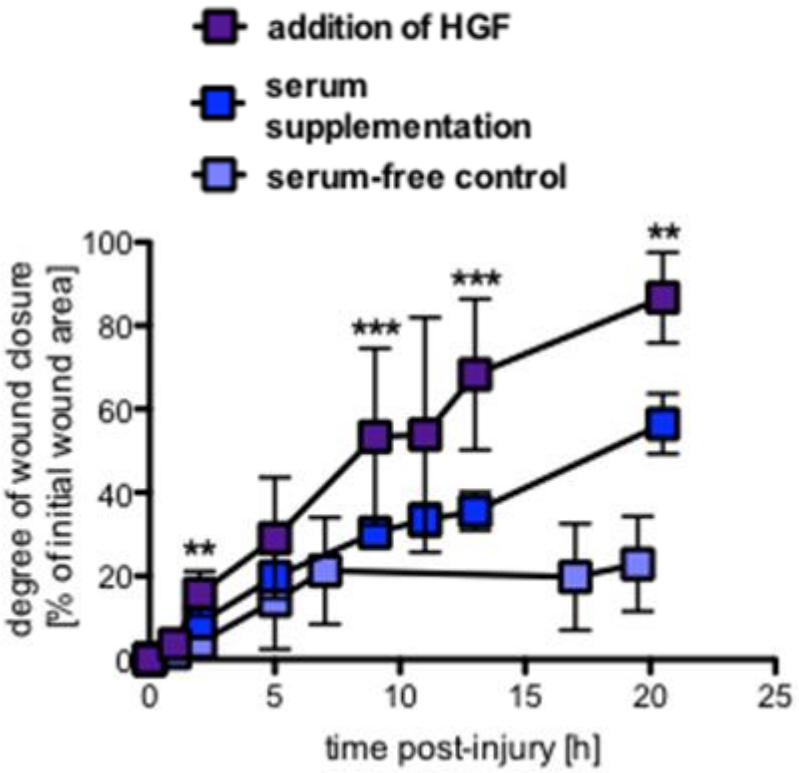
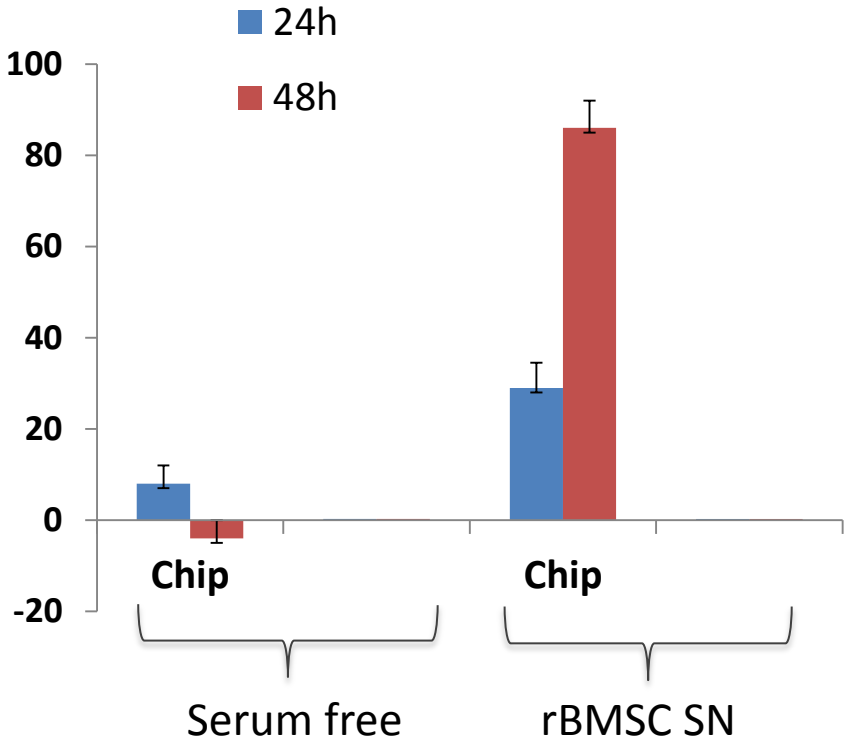
HGF exposure



Felder et al, Lab Chip, 2012

rBMSC SN exposure



Marconi et al, submitted

Wound-healing in a microfluidic channel

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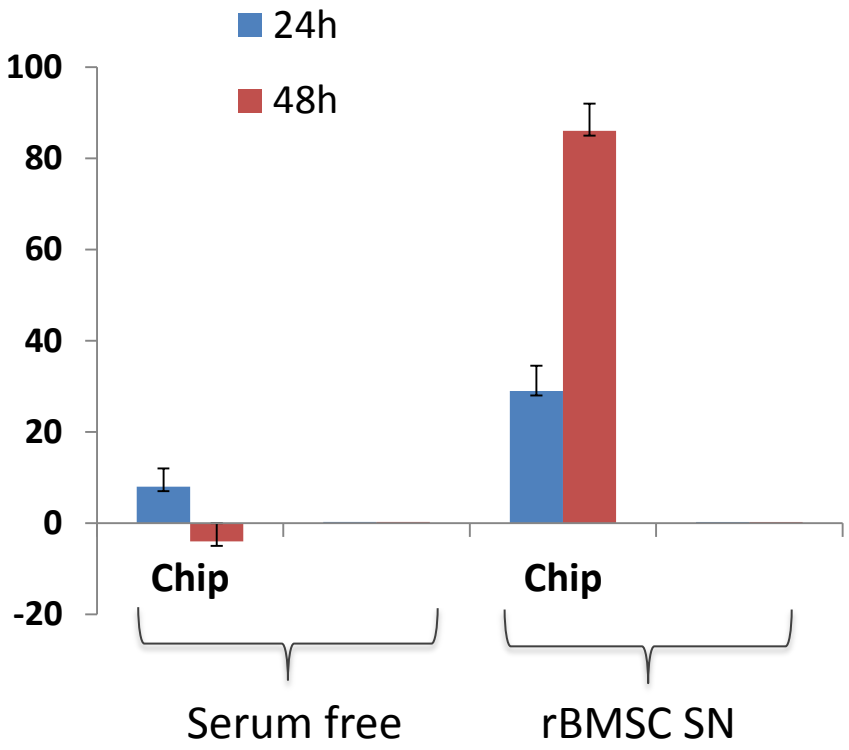
rBMSC SN exposure

Perfused system (chip):

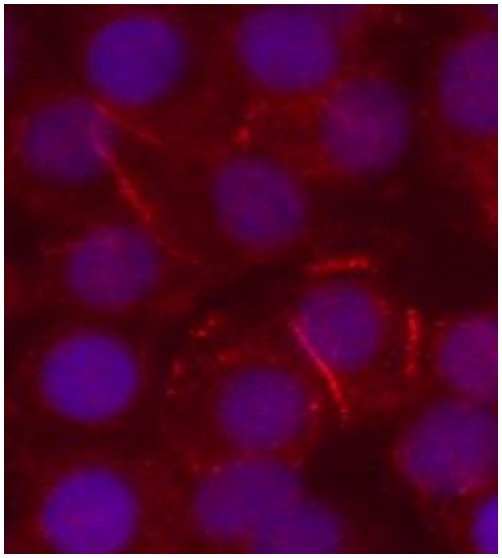
- Smaller epithelial wound
- Small amount of cells
- Constant Renewal of:
  - Nutrients
  - Oxygen
- Constant Removal of:
  - Cytokine
  - Cellular waste

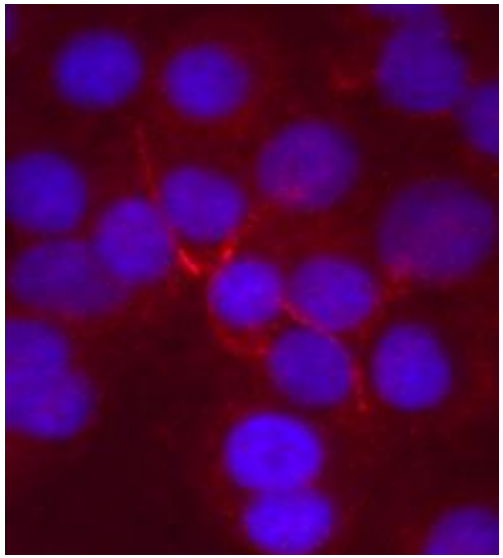


Marconi et al, submitted

Preservation of alveolar type II phenotype

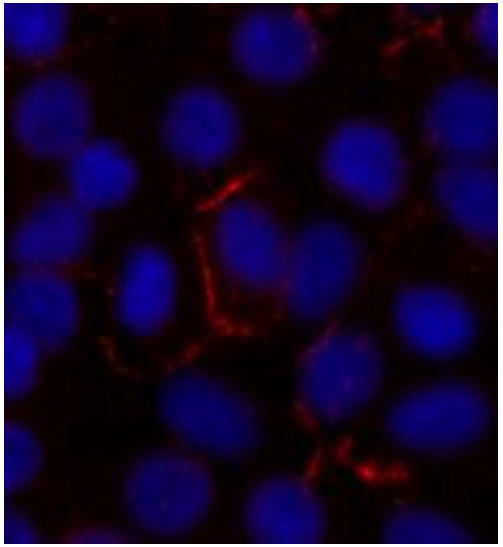
Epithelial tight junctions (occludin immunostaining and cell nuclei DAPI)

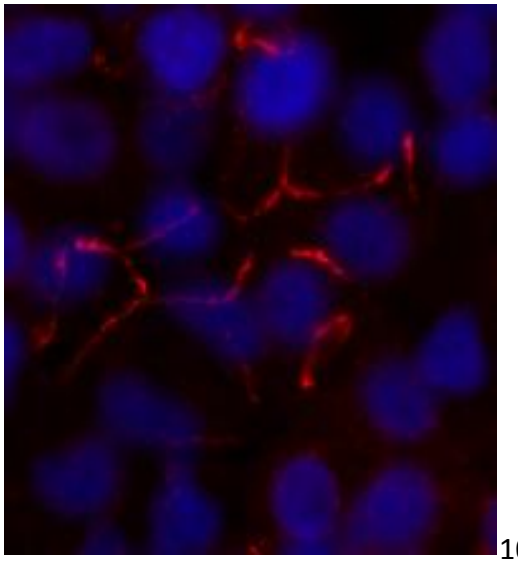




A549 cells

Epithelial tight junctions
ZO-3 expressions and cell nuclei (DAPI)

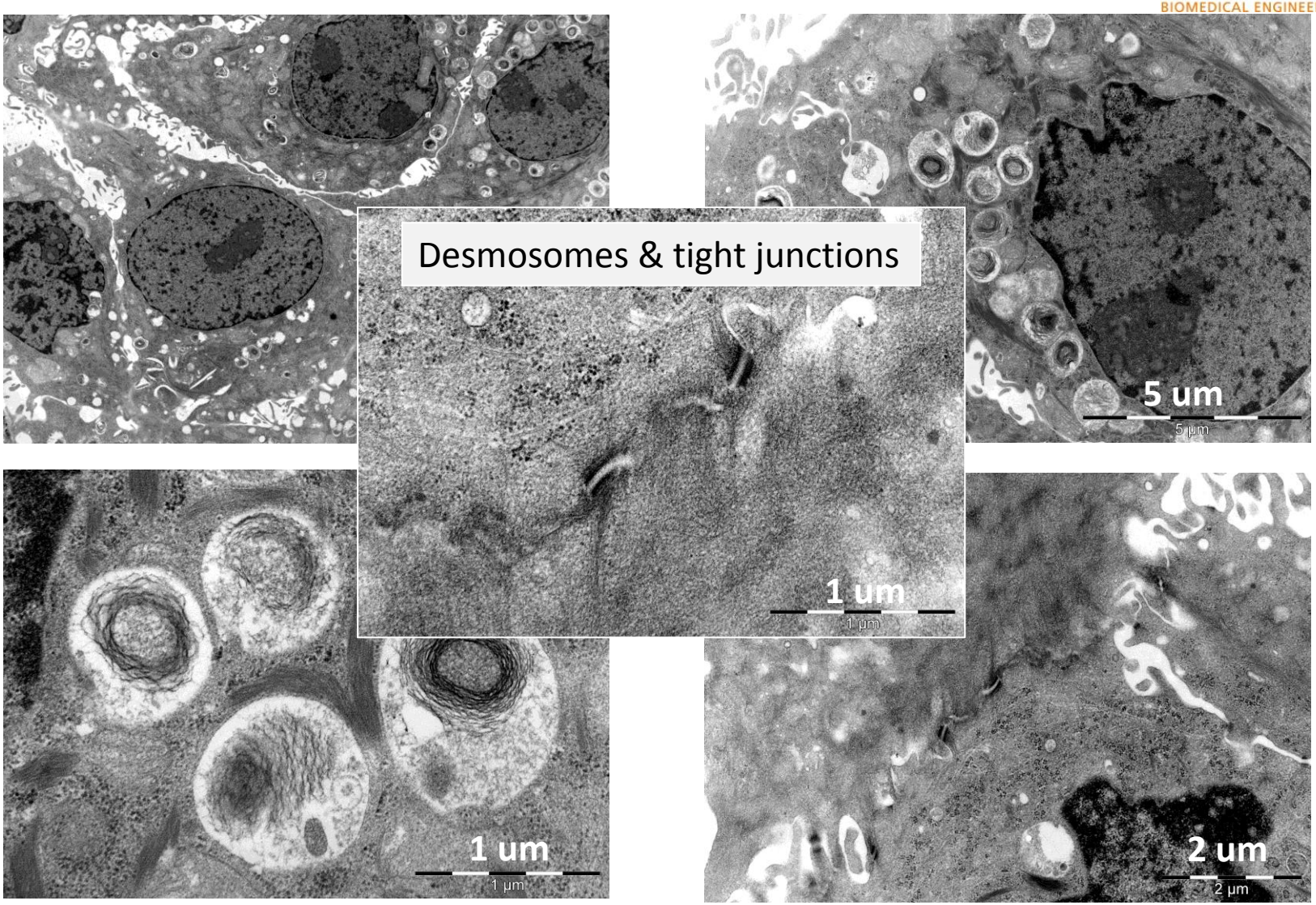




Felder et al, Lab Chip, 2012

Preservation of alveolar type II phenotype

Lamellar bodies: production of surfactant



Felder et al, Lab Chip, 2012

Towards in-vitro models of pulmonary diseases

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ARTORG Lung Lab objectives: recreate in-vitro the in-vivo conditions of the lung

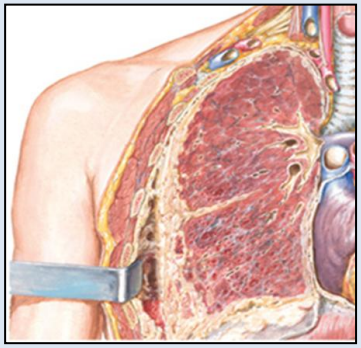
Pulmonary Fibrosis:

Median survival 3yrs
> 45'000 cases/year (US)
Prevalence (+150% since 2001)

No effective treatment

Repeated microinjuries of lung epithelial layer with abnormal wound repair

Malignant Pleural Mesothelioma (MPM):



rare disease: 160 cases/year (CH)
median survival: +/- 12 months

No effective treatment

60-70% cases due to asbestos
Main problem: disease recurrence

Improving in-vitro models of MPM

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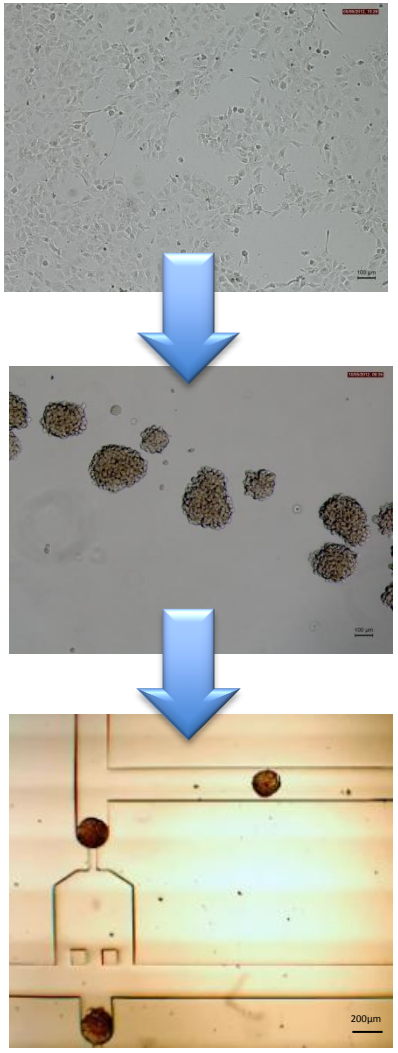

Standard Culture Monolayer (2D)
2D system
Static model

Standard Culture Spheroids (3D)
3D architecture
Static model

Spheroids (3D) on Chip
3D architecture
Perfused model
Accurate drug delivery

Chemosensitivity platform for MPM spheroids

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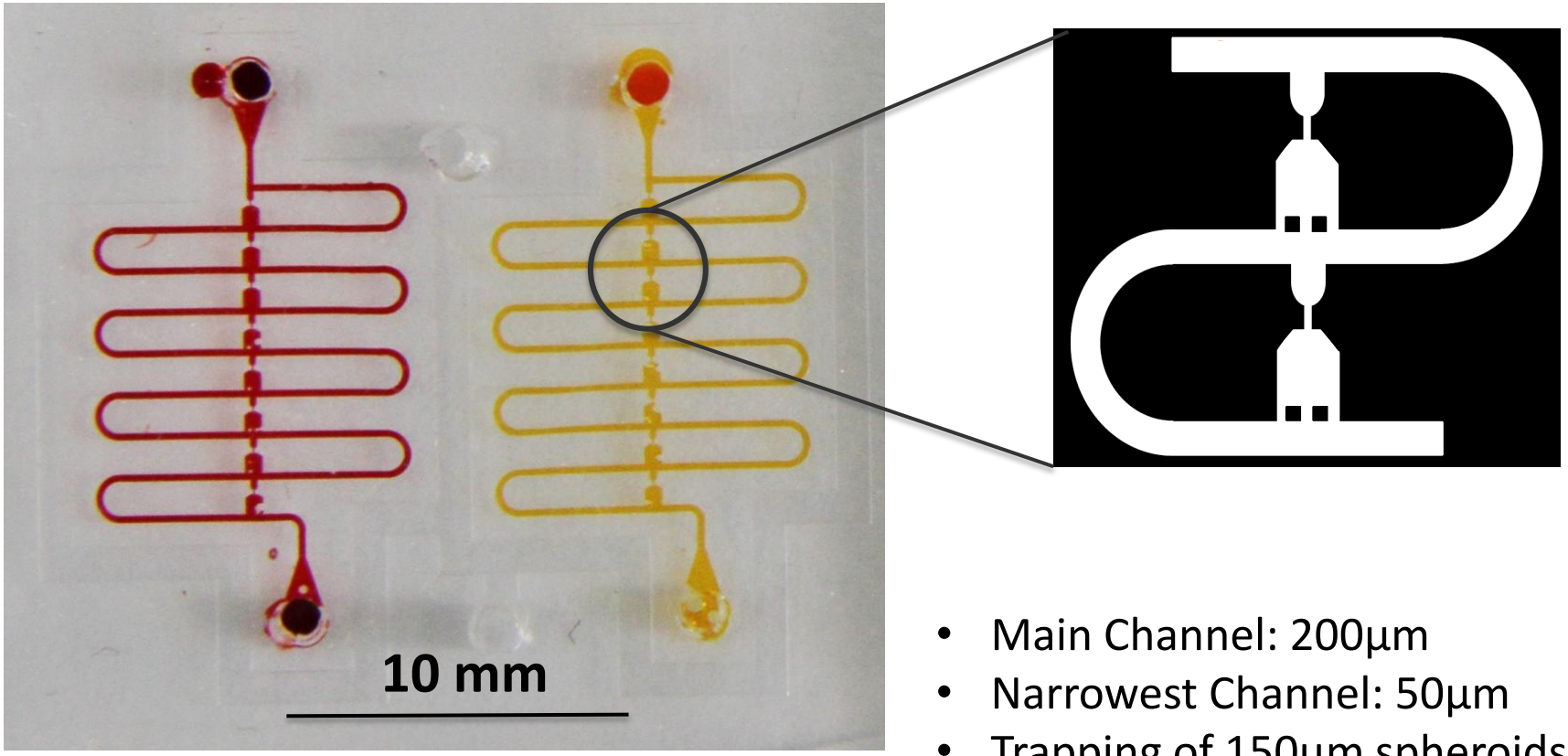
- Need to develop a technology to trap and perfuse small spheroids (∅150um)
- Develop read-out protocols that can be compared to standard assays



- Main Channel: 200µm
- Narrowest Channel: 50µm
- Trapping of 150µm spheroids

Ruppen et al. , British Journal of Surgery 2012; 99 (Suppl. 5): 1–24

Trapping spheroids on chip

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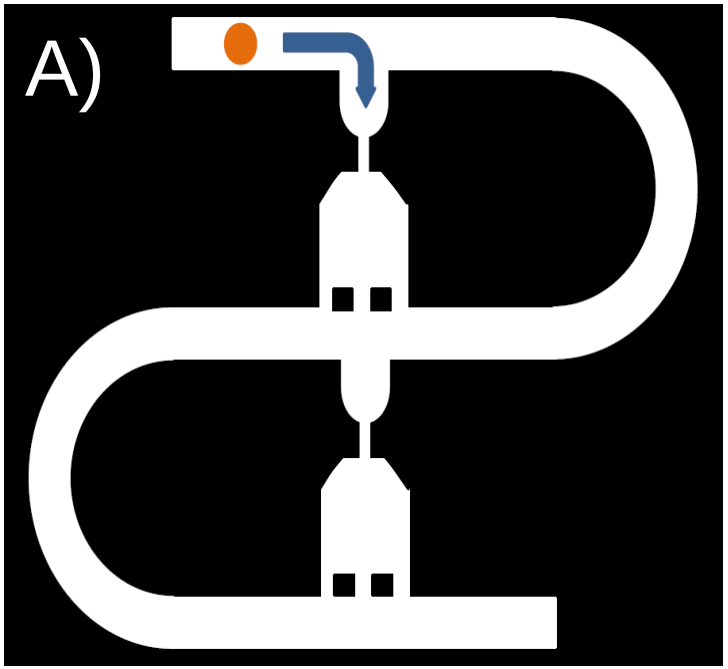
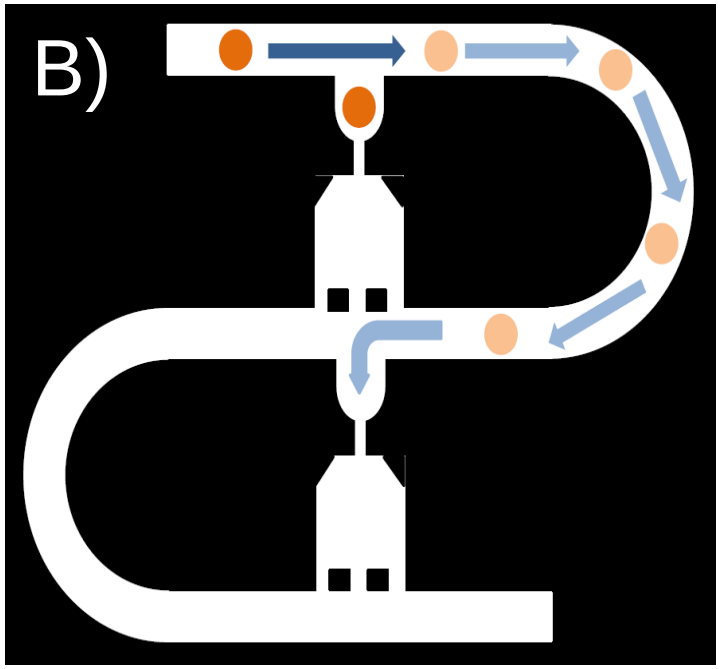
Trapping principle: smallest hydraulic resistance path[1]



Empty Trap



Trap with Spheroid

[1] Tan, Takeuchi, PNAS, 2007 (beads trapping)

Trapping of 150um in diameter MPM spheroids on chip

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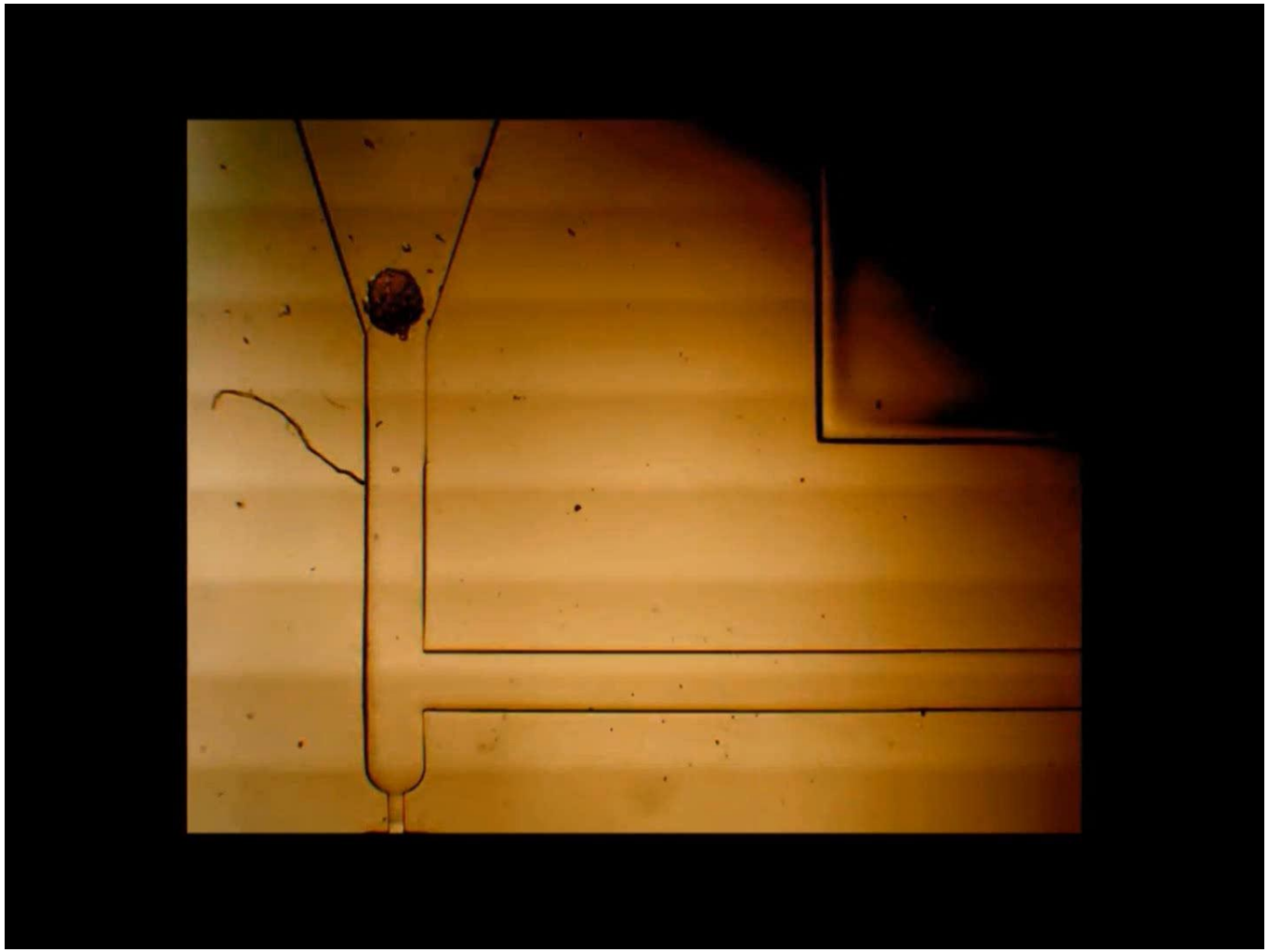

Ruppen et al. , British Journal of Surgery 2012; 99 (Suppl. 5): 1–24

IC_{50} of Cisplatin for different *in vitro* models for MPM (H2052)

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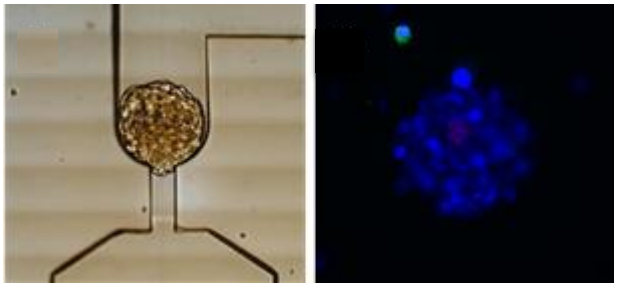
IC50 [µM]	XTT	Hoechst/PI
2D	32	45
3D	128*	125
3D Chip	-	320*



* Preliminary Data

$$(IC_{50})_{2D} \overset{4x}{<} (IC_{50})_{3D} \overset{2x}{<} (IC_{50})_{CHIP}$$

Summary and outlook

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- Novel in-vitro models of the lung are needed
- Microfluidics
  - Perfusion (maintained phenotype)
  - Epithelial microinjuries (5x smaller than scratch test)
  - Trapping 3D tissue for chemosensitive assay
  - Accurate drug delivery
  - Use of small cell number (personalised medicine)
  - Mimic the respiratory movements

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