

PATROLS

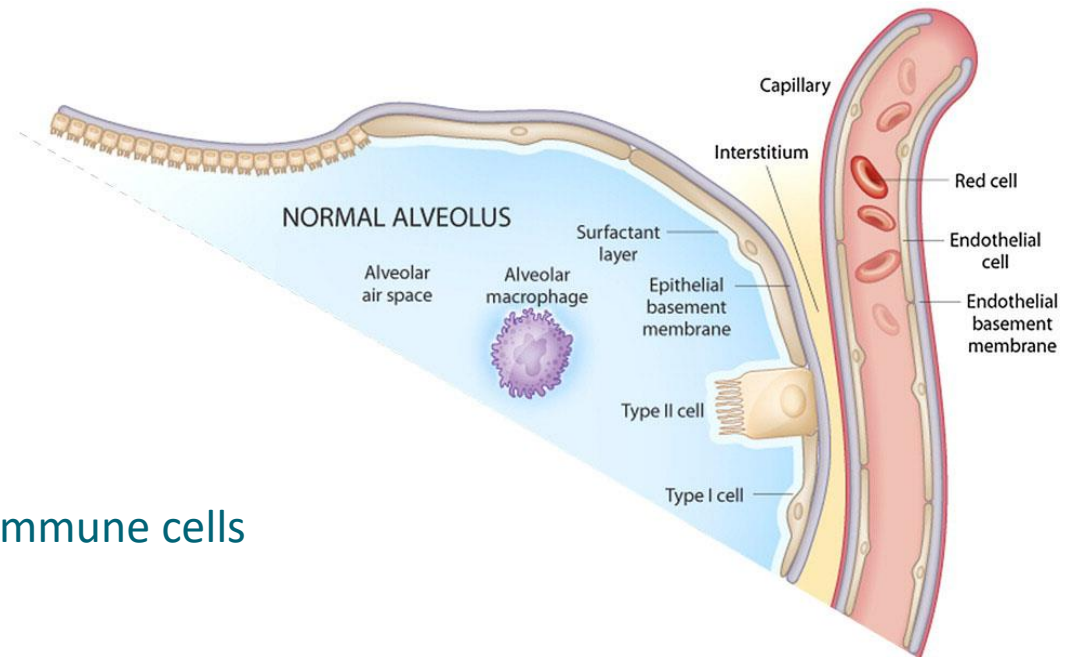
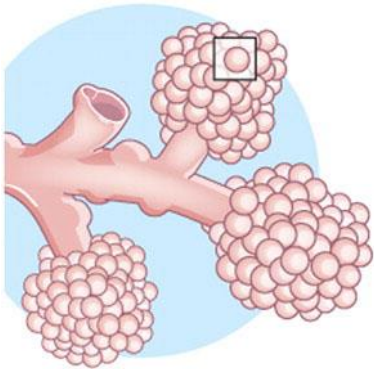
Advanced Tools for NanoSafety Testing

Induction of inflamed alveolar epithelial model for nanomaterial hazard assessment

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Co-culture model to mimic the human alveolar epithelium



Triple cell co-culture model:
Epithelial cells + two types of immune cells



Air-liquid interface

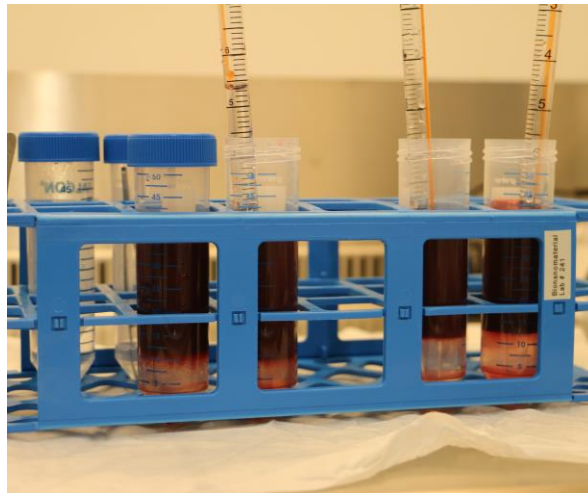
Cell culture medium ↔ circulatory system

Isolation of peripheral blood derived monocytes

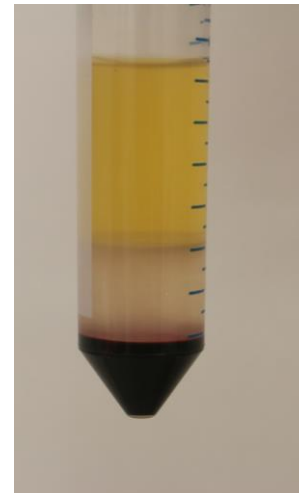
Purification of human mononuclear cells:
LymphoPrep™ (Stemcell™ technologies)



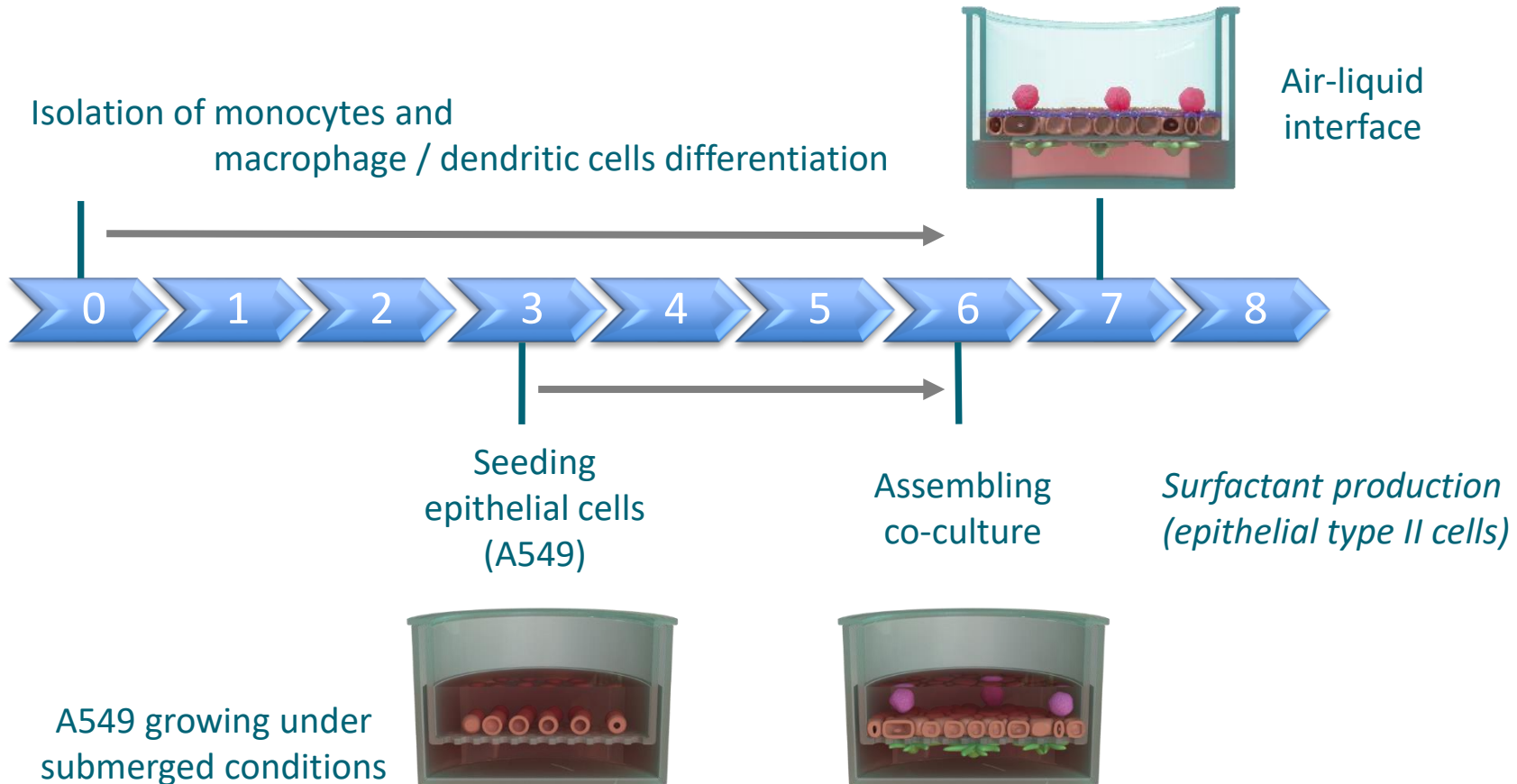
INTERREGIONALE BLUTSPENDE SRK
TRANSFUSION INTERREGIONALE CRS



Isolation of monocytes by magnetic
activated cell sorting (anti-human CD14)

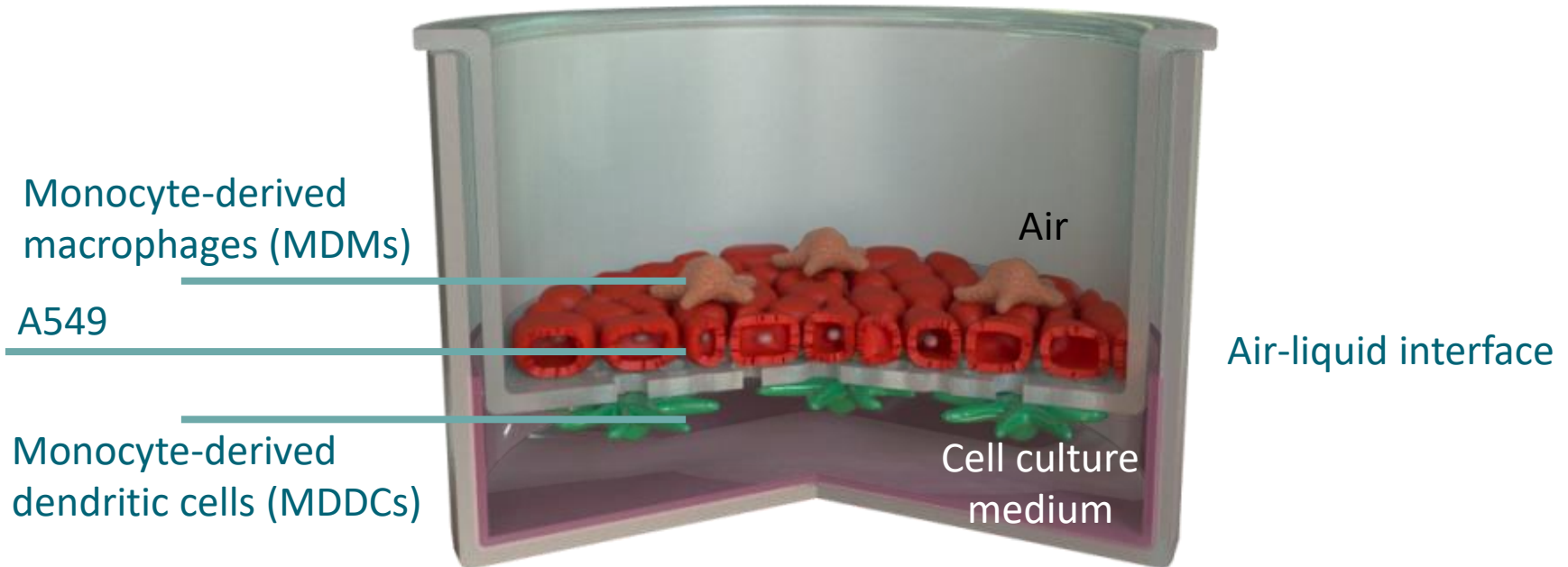


Composition of the alveolar epithelial model



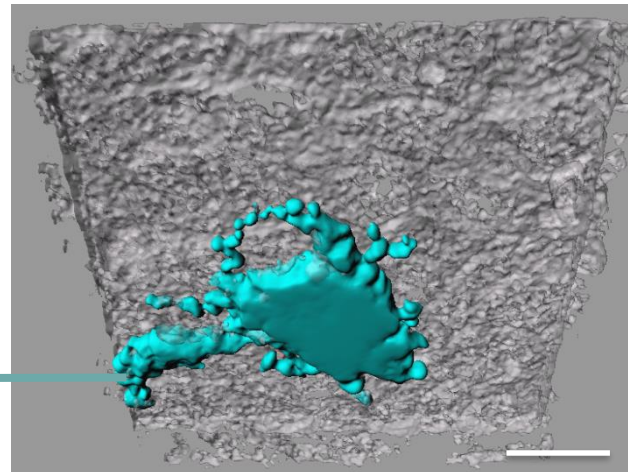
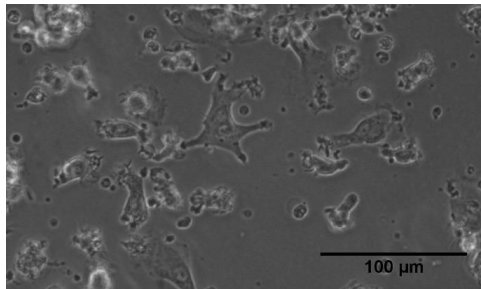
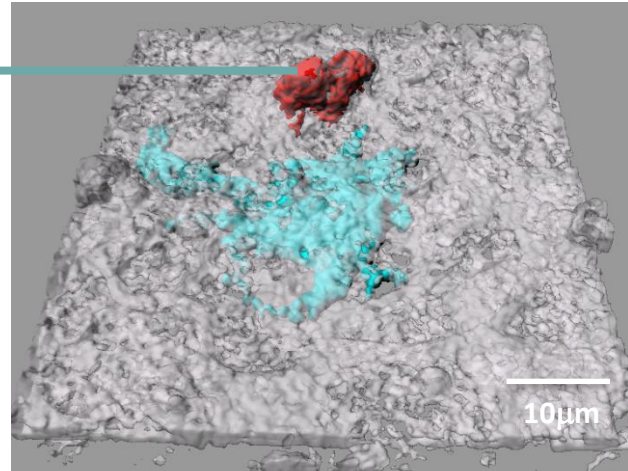
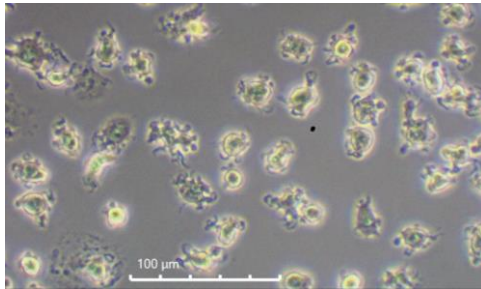
Alveolar epithelial barrier model: construction

Epithelial cells (A549 cells representing epithelial type II cells) +
two types of primary immune cells (differentiated from peripheral blood derived monocytes)



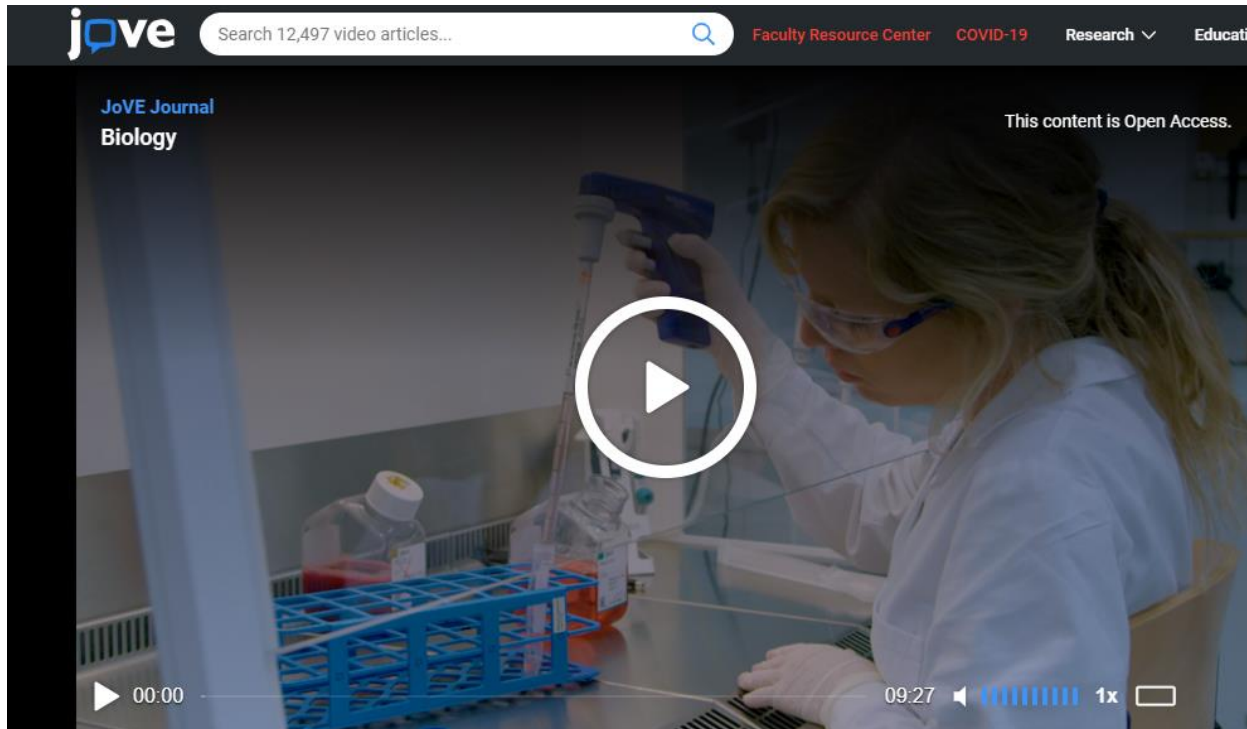
Alveolar epithelial barrier model: construction

Monocyte-derived macrophages (MDMs)



Monocyte-derived dendritic cells (MDDCs)

Video protocol available online

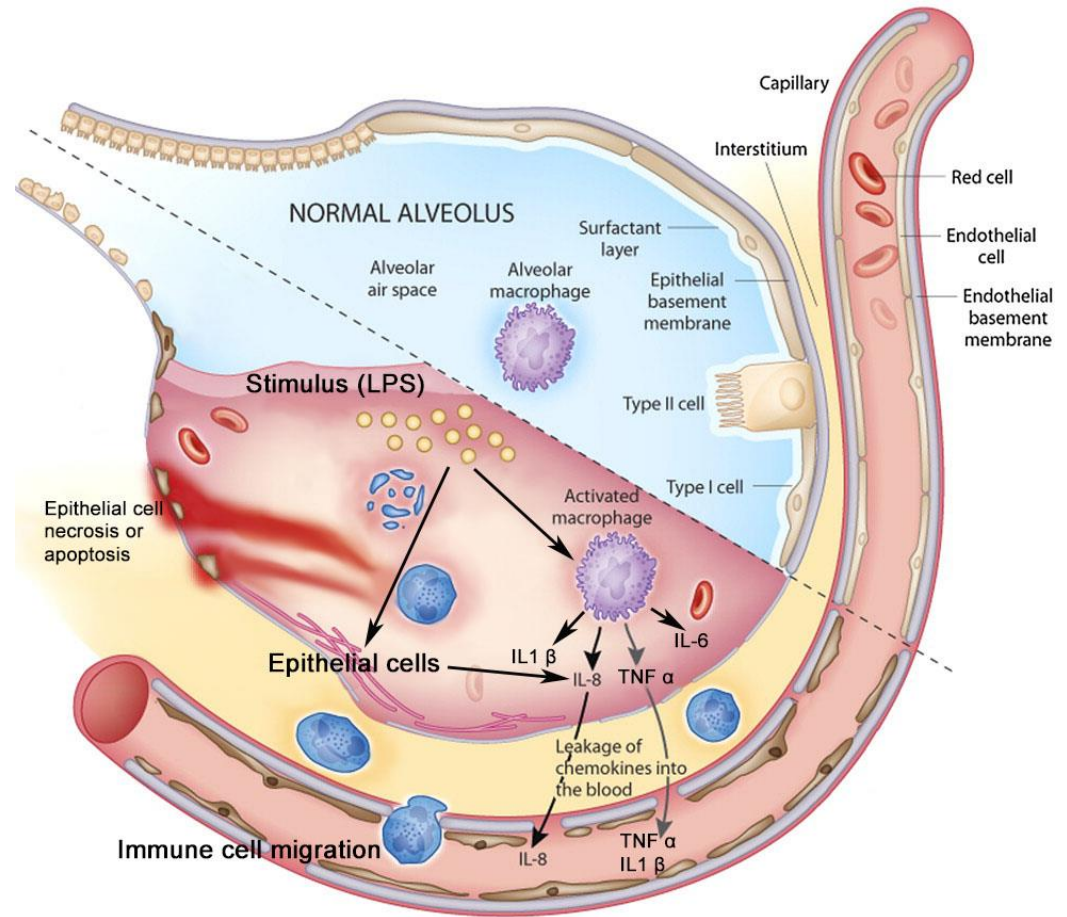
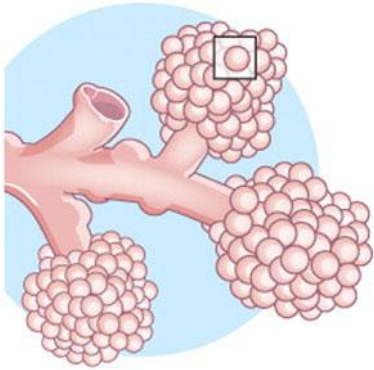


Multicellular Human Alveolar Model Composed of Epithelial Cells and Primary Immune Cells for Hazard Assessment

doi: 10.3791/61090

<https://www.jove.com/v/61090/multicellular-human-alveolar-model-composed-epithelial-cells-primary>

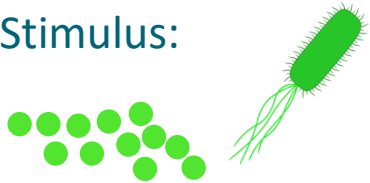
Alveolar inflammation: a symptom of various lung diseases and infections



Alveolar inflammation:
affects O_2 / CO_2
exchange

Pro-inflammation in the tissue: “state of alert”

Stimulus:

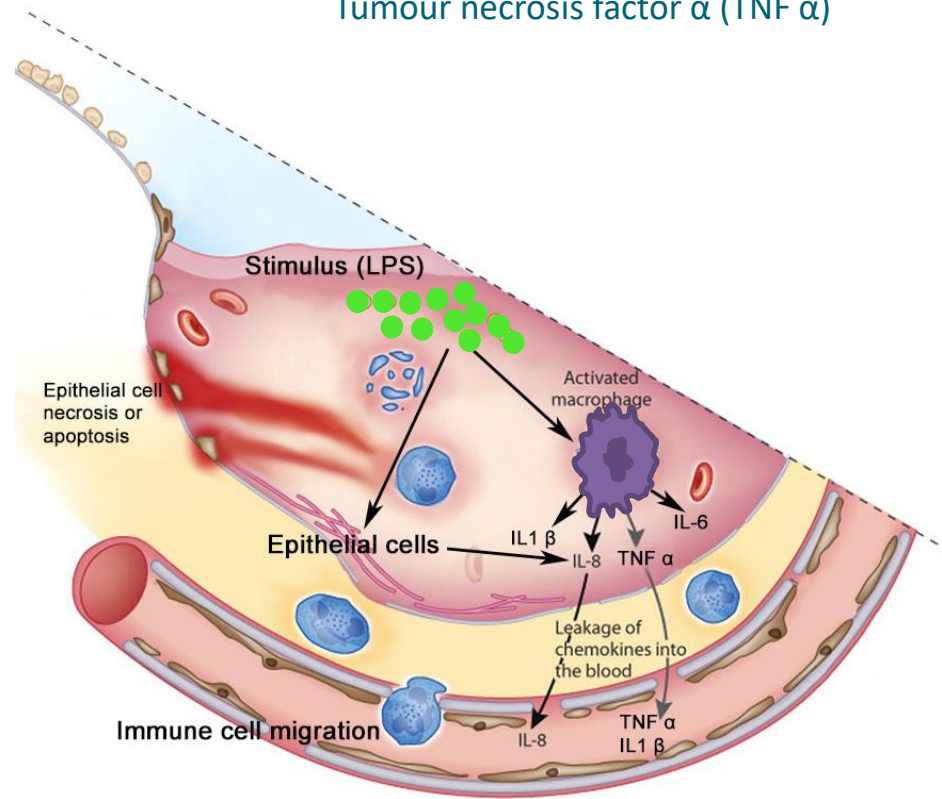


Lipopolysaccharide (LPS), a bacterial cell wall component

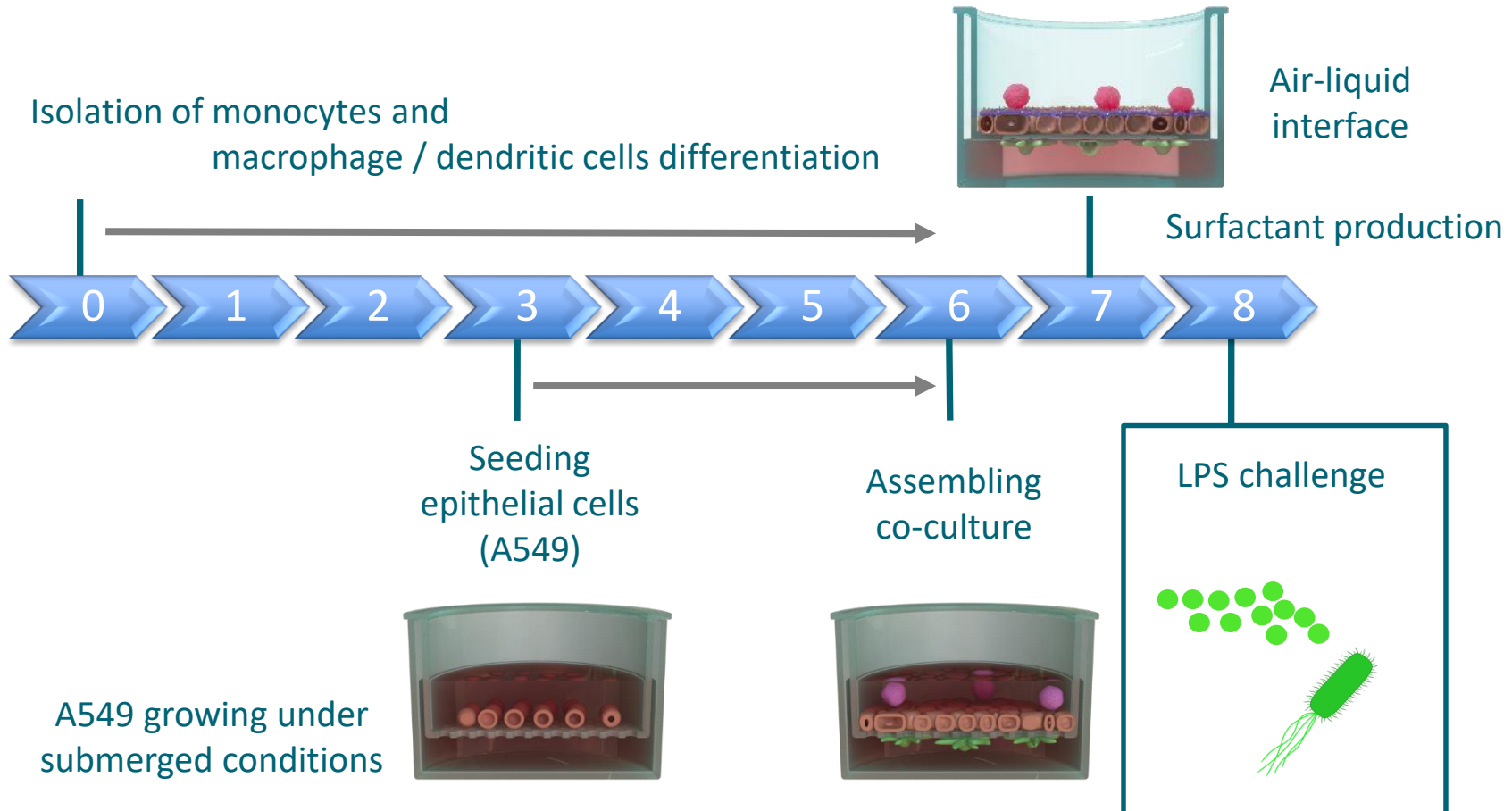
Attracting immune cells from circulation

Response: Release of pro-inflammatory cytokines

e.g. Interleukin 8 (IL 8)
Tumour necrosis factor α (TNF α)

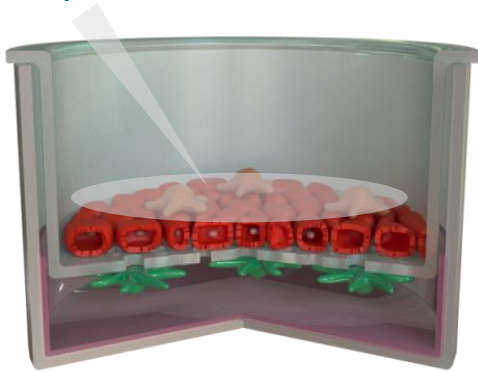


Inflamed model induction: LPS challenge



Inflamed model: induction, features and *in vitro* tools

LPS apical side:
as quasi-ALI



LPS basal side:
in cell culture medium

Lipopolysaccharide (LPS):
1 µg/mL in cell culture medium

1. Compromised barrier integrity

- Visualization: confocal microscopy
- Permeability tests, *e.g.* fluorescein

2. Pro-inflammation

Pro-inflammatory markers, *e.g.* interleukin 8, tumour necrosis factor α , interleukin 1 β

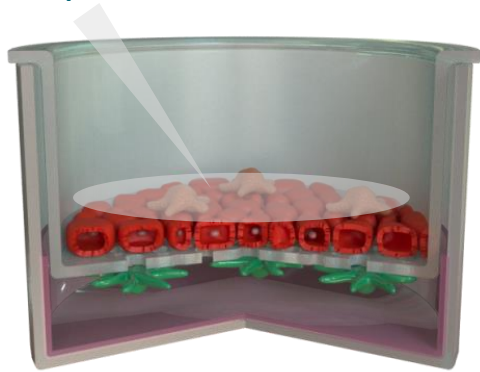
- Secretion: ELISA
- Gene expression: PCR

3. Various endpoints

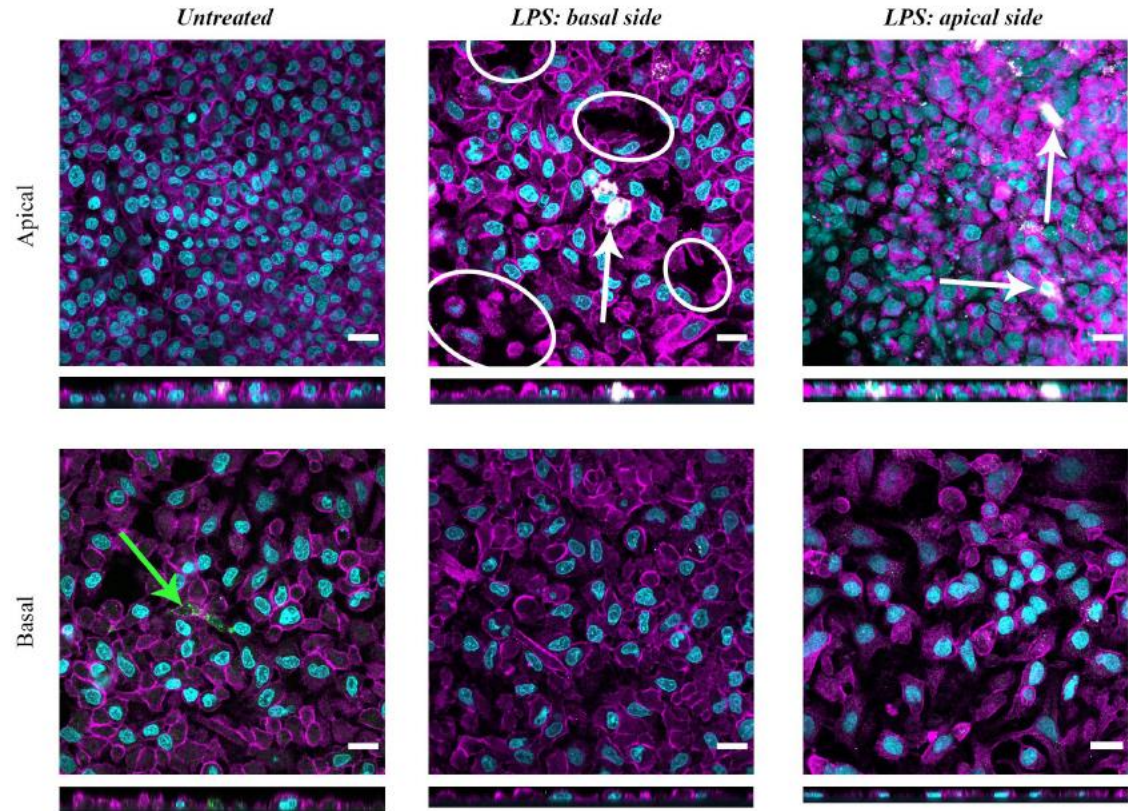
- Cytotoxicity
- Oxidative stress
-

LPS challenge optimization: basal side challenge for 48 h

LPS apical side:
as quasi-ALI

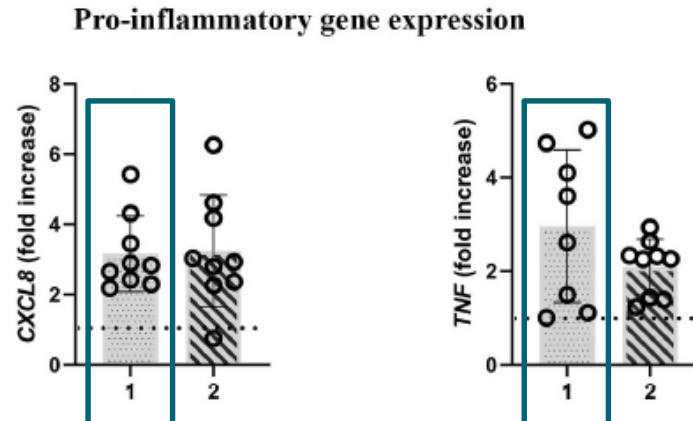
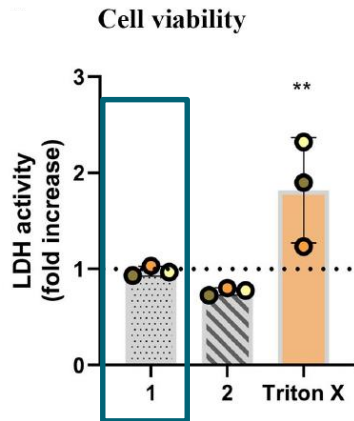
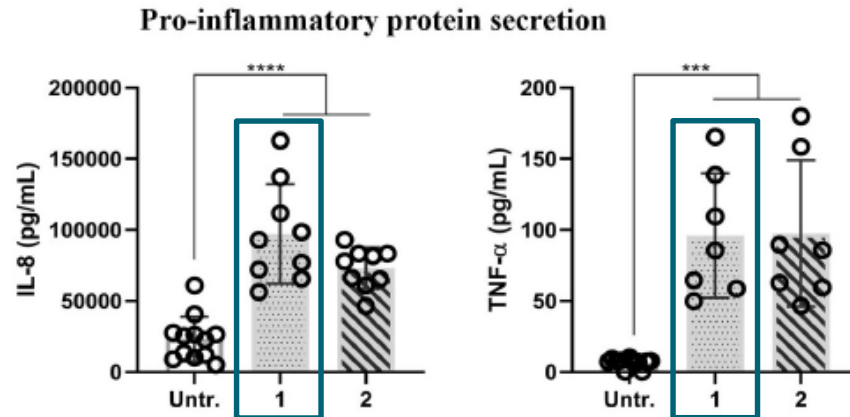
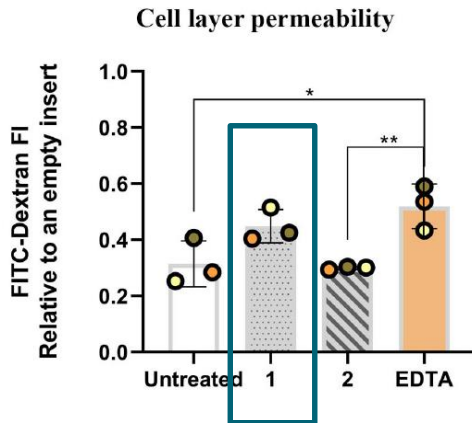


LPS basal side:
in cell culture medium



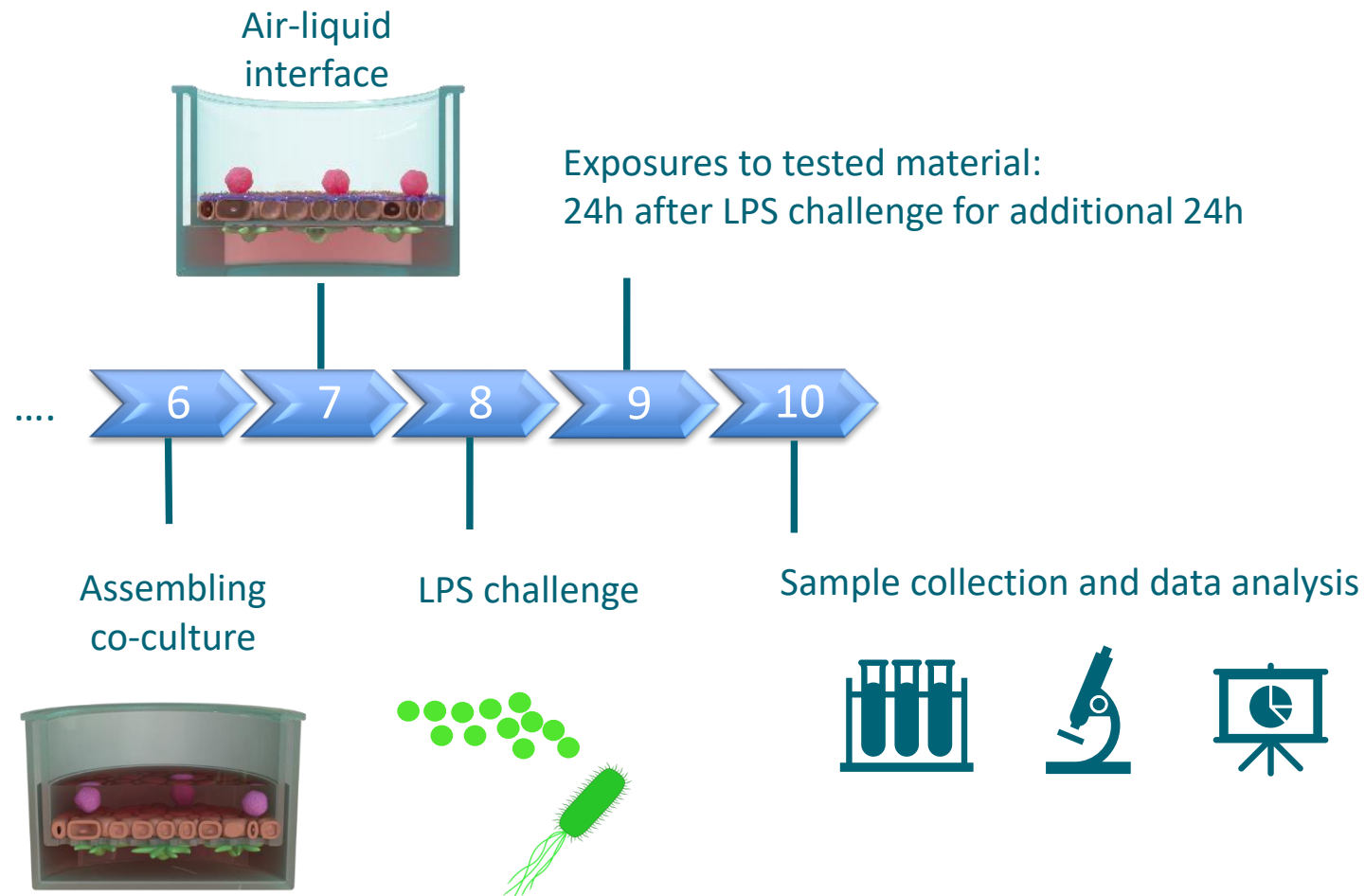
Staining: nuclei (DAPI; cyan), cytoskeleton (rhodamine-phalloidin; magenta), macrophages (mature macrophage marker 25F9; white + arrows), dendritic cells (CD83; green + arrow). Ellipsoids: regions with epithelial barrier disruption. Scale bars: 20 μ m.

LPS challenge from the basal side shows inflamed tissue characteristics

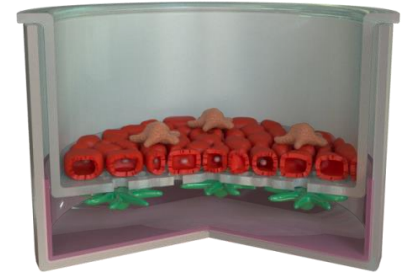


1 – basal side, 2 – apical side

Inflamed model ready for exposures to nanomaterials



Take home messages



- 10-day protocol:
 - ✓ Cell model assembly, pro-inflammation induction, exposure to nanomaterials
 - + Analysis
 - Lipopolysaccharide (LPS) is used as a pro-inflammatory stimulus *in vitro*
 - The main characteristics of inflamed model (can be mimicked *in vitro*):
 - ✓ Impair barrier integrity
 - ✓ Pro-inflammatory markers
- } Tools: Confocal microscopy, permeability tests
ELISA and PCR (more sensitive)

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adolphe merkle institute
excellence in pure and applied nanoscience

PATROLS consortium, in particular WP3 partners

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