



Epithelix

Provider of human tissues reconstituted in vitro

MucilAir: a Novel Human 3D Airway Epithelium Model for Long Term Toxicity Testing

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Epithelix Sàrl

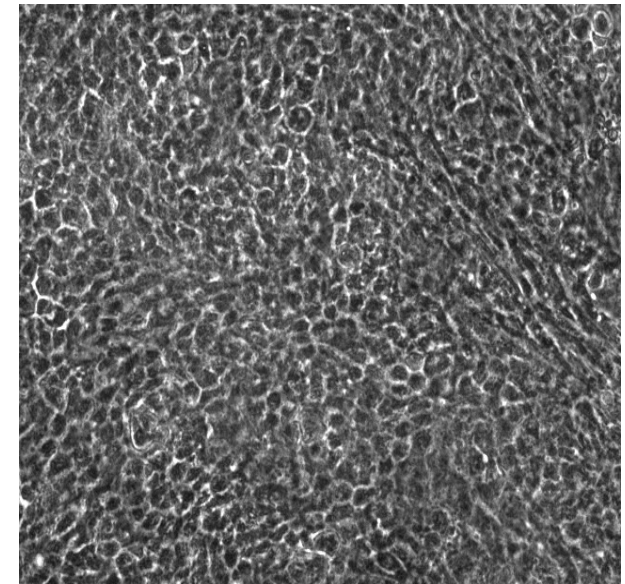
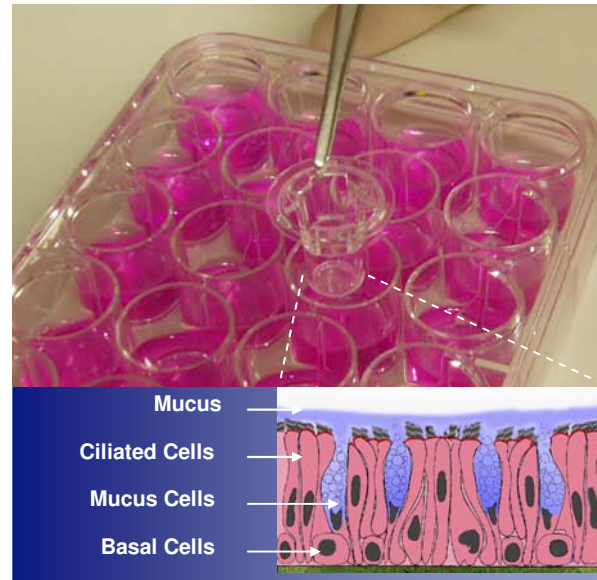
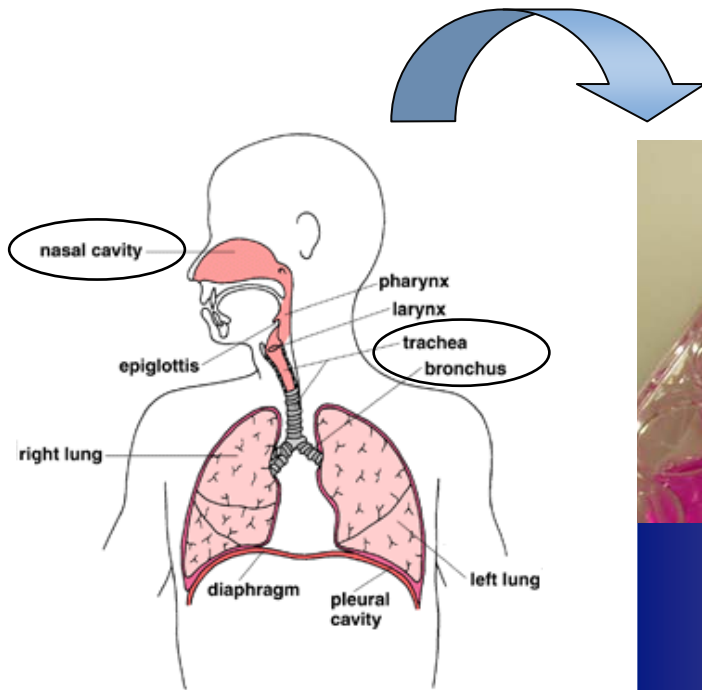
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Isolation, amplification & seeding
Primary Human Cells

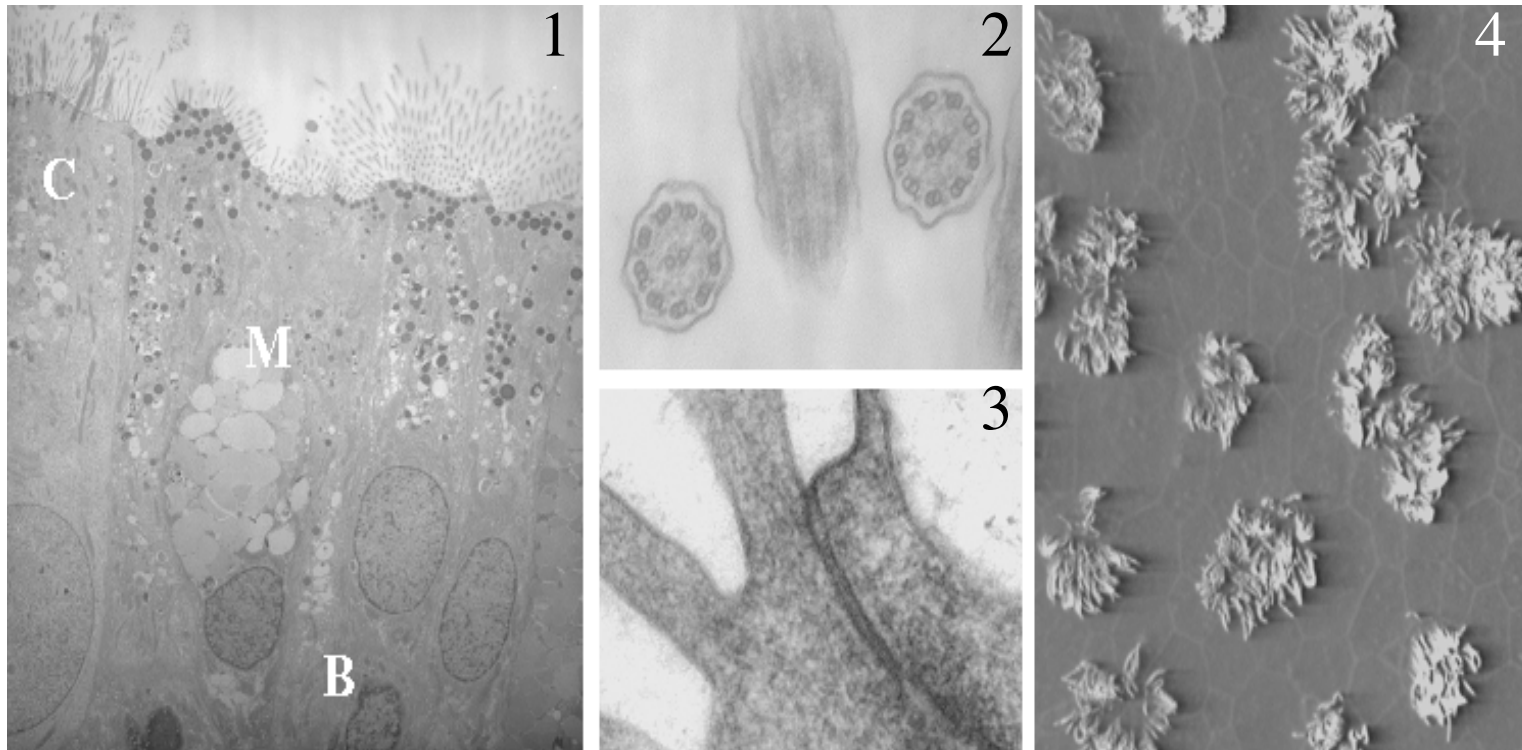
Ready-to-use
Fully differentiated Epithelium



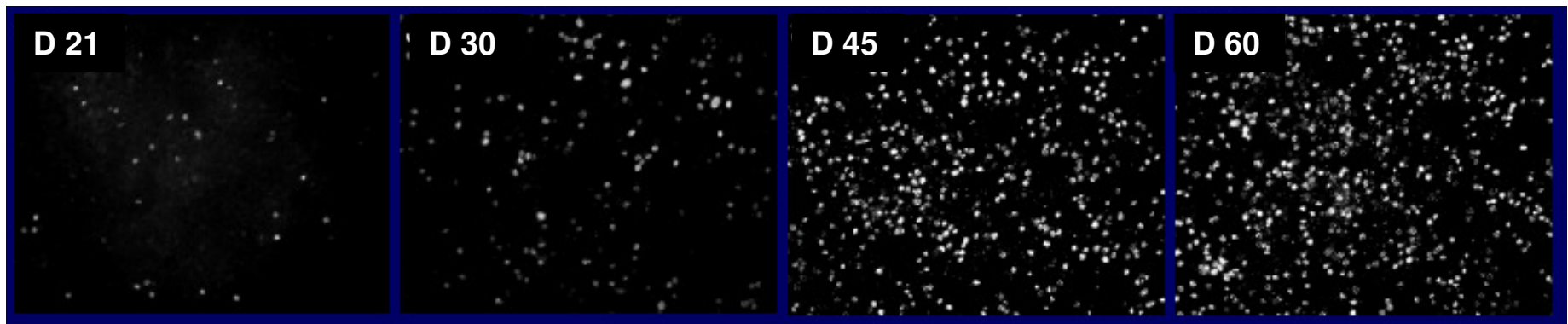
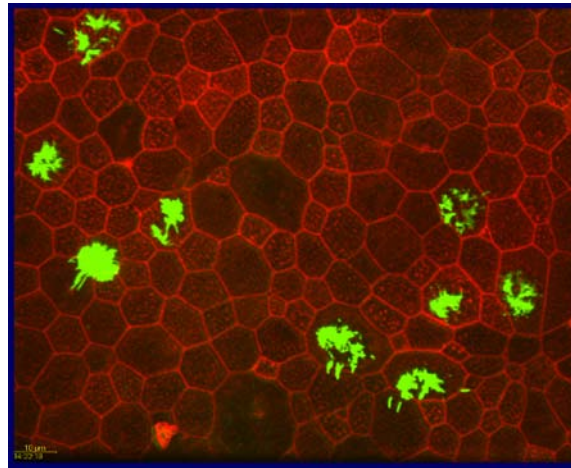
- Patients
 - ✓ Normal
 - ✓ Asthmatic
 - ✓ Smokers
 - ✓ COPD
 - ✓ CF

Air-interface, differentiation

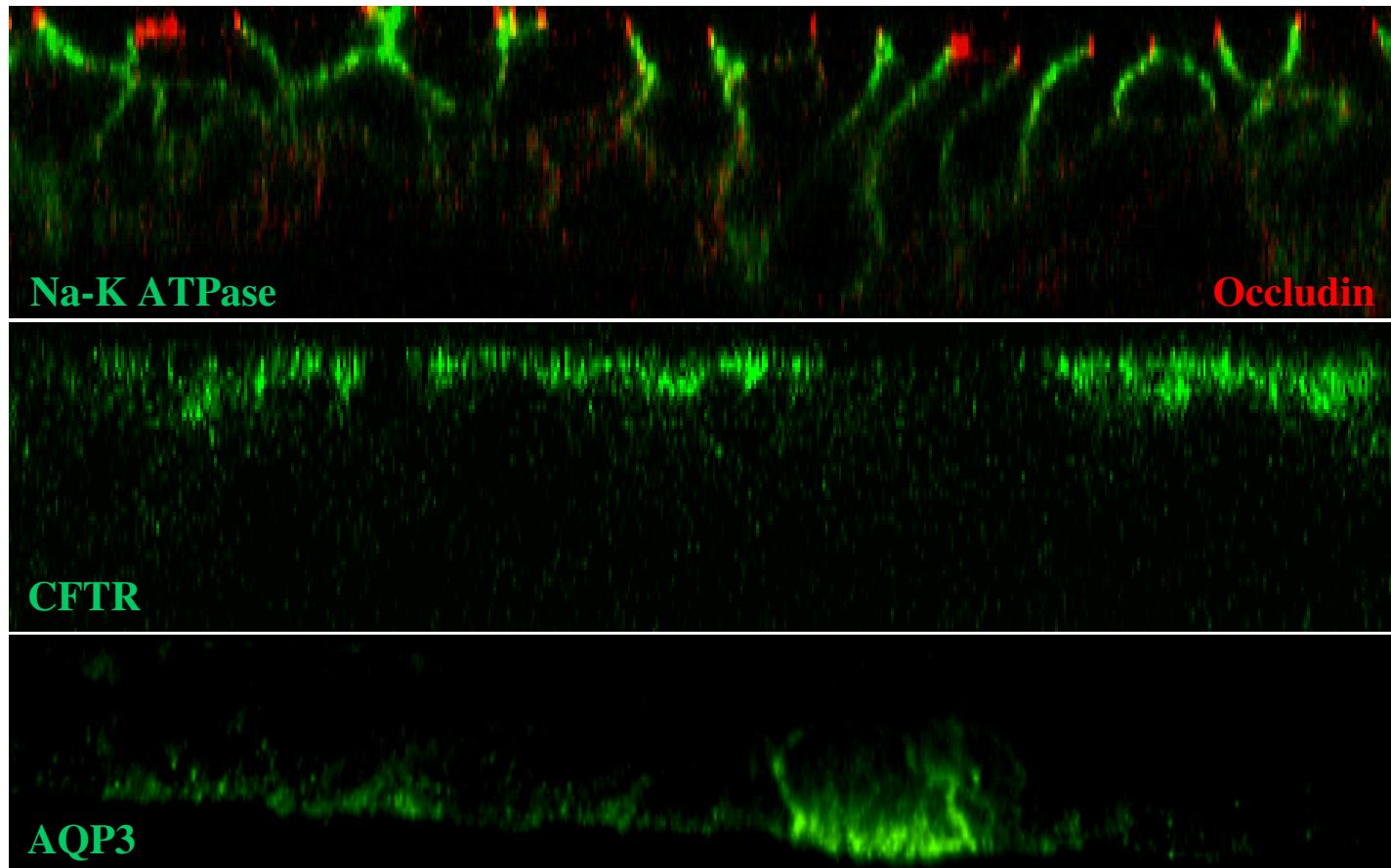
Similar, if not identical, ultra-structures as in the native tissues



A time course of cilia formation monitored by anti- β -tubulin antibody



Various ions channels and cell markers are detected in our *in vitro* model of airway epithelium





- It closely **mimics** the morphology and functions of the normal human airway epithelium.
- It has a unique shelf-life of **one year**.
- It is **easy** to handle and maintain.
- Adapted to **high-throughput** screening and testing.
- Serum free.

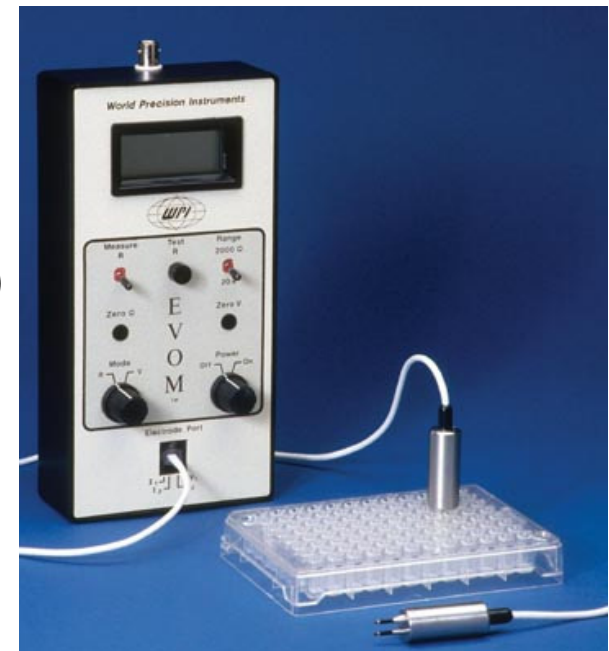
Applications of MucilAir



- Toxicity Testing (cell viability and tissue integrity)
- Effect on ciliogenesis
- Effect on cilia beating (mucociliary clearance)
- Effect on Mucin secretion
- Measurement of pro-inflammatory mediators
- Simulation of chronic inflammation
- Electrophysiological activity (TEER, ion channels)
- Drug transport, etc.

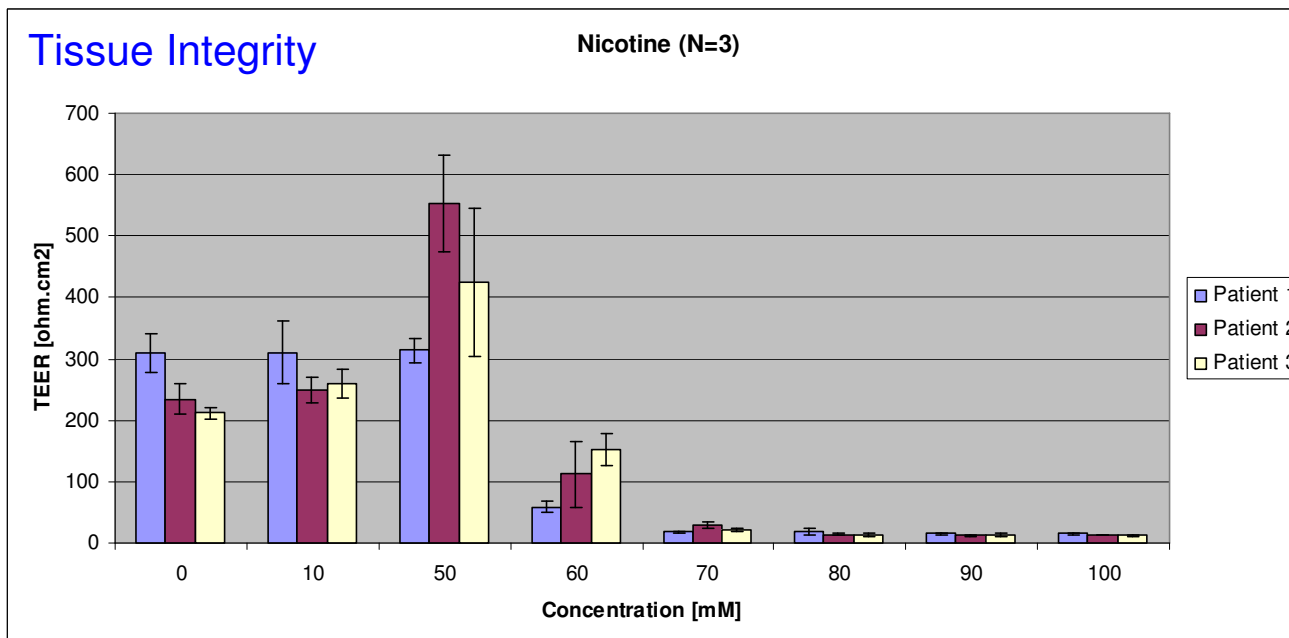
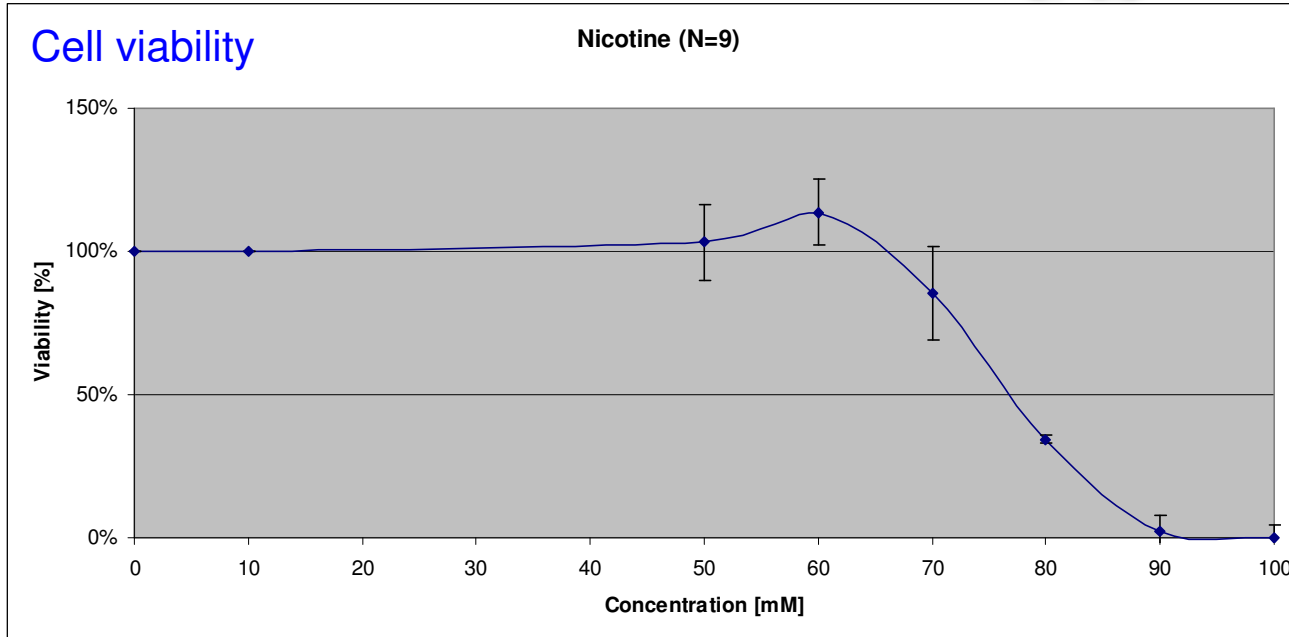
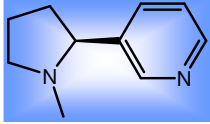
MucilAir is a powerful tool to quantify Acute, Chronic and Long Term effect of molecules

- Cell viability (EC_{50} & ET_{50})
 - ✓ Resazurin test (Quantitative)
- Tissue Integrity
 - ✓ Trans Epithelial Electrical Resistance (Quantitative)
- Cilia beating Monitoring
 - ✓ Visual Inspection (Qualitative)
- Time points
 - ✓ Acute = 1 hour exposure
 - ✓ Chronic = Multiple 1 hour exposures
 - ✓ Long Term exposure (e.g. 24h, 48h, etc.)
- Type of Compounds
 - ✓ Solid, liquid
 - ✓ Gas, nanoparticles, smoke: Cultex Chambers

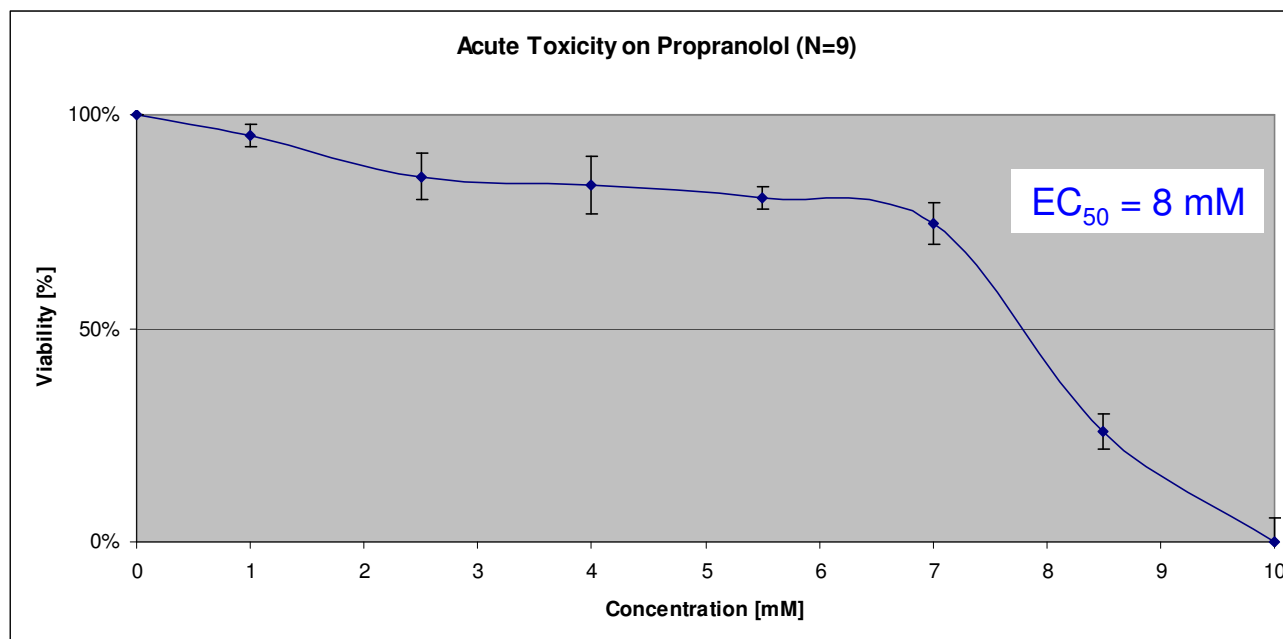
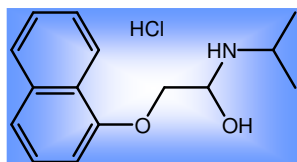


CULTEX®

Acute Toxicity (1 hour Exposure)



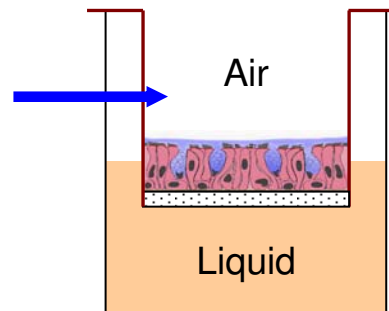
Chronic Toxicity (Multiple 1 hour Exposures)



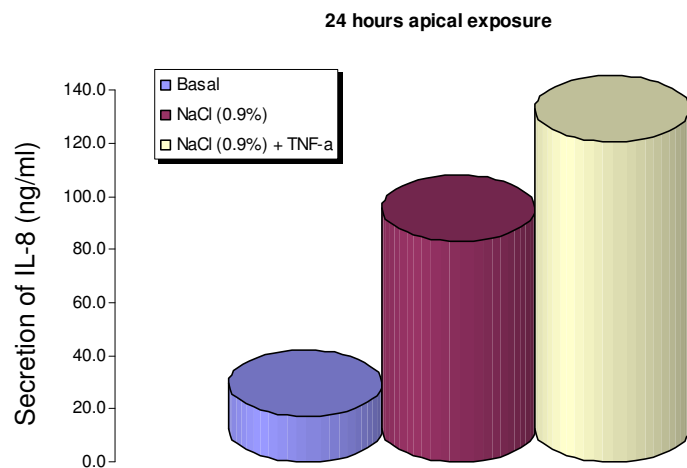
Concentrations	0 mM	1 mM	2.5 mM	4 mM	5.5 mM	7 mM	8.5 mM	10 mM
Expo 1 (Day 0)	✓	✓	✓	✓	✓	✓	EC ₂₅	☠
Expo 2 (Day 7)	✓	✓	✓	✓	✓	✓	☠	☠
Expo 3 (Day 14)	✓	✓	✓	✓	✓	☠	☠	☠
Expo 4 (Day 21)	✓	✓	✓	✓	✓	☠	☠	☠
Expo 5 (Day 28)	✓	✓	✓	✓	☠	☠	☠	☠
Expo 6 (Day 35)	✓	✓	✓	✓	☠	☠	☠	☠

2 exposure modes

Apical exposure

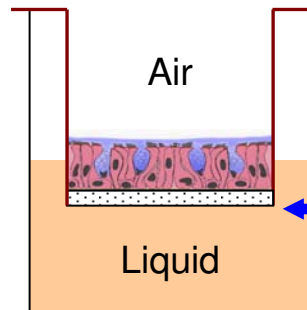


- Ideal for gas, particles.
- Typical reaction with liquids :



2 exposure modes

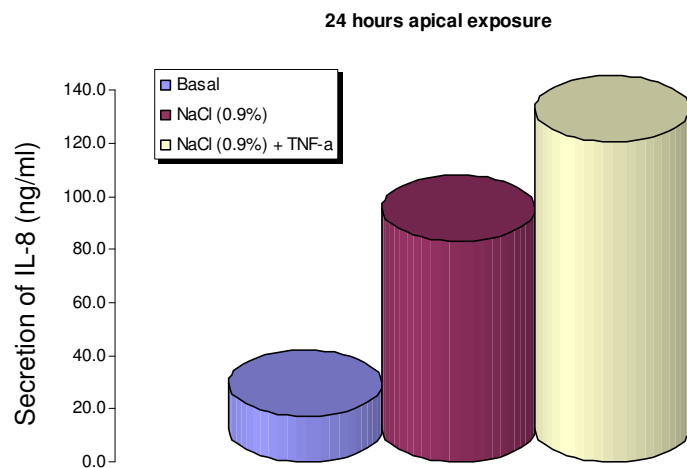
Apical exposure



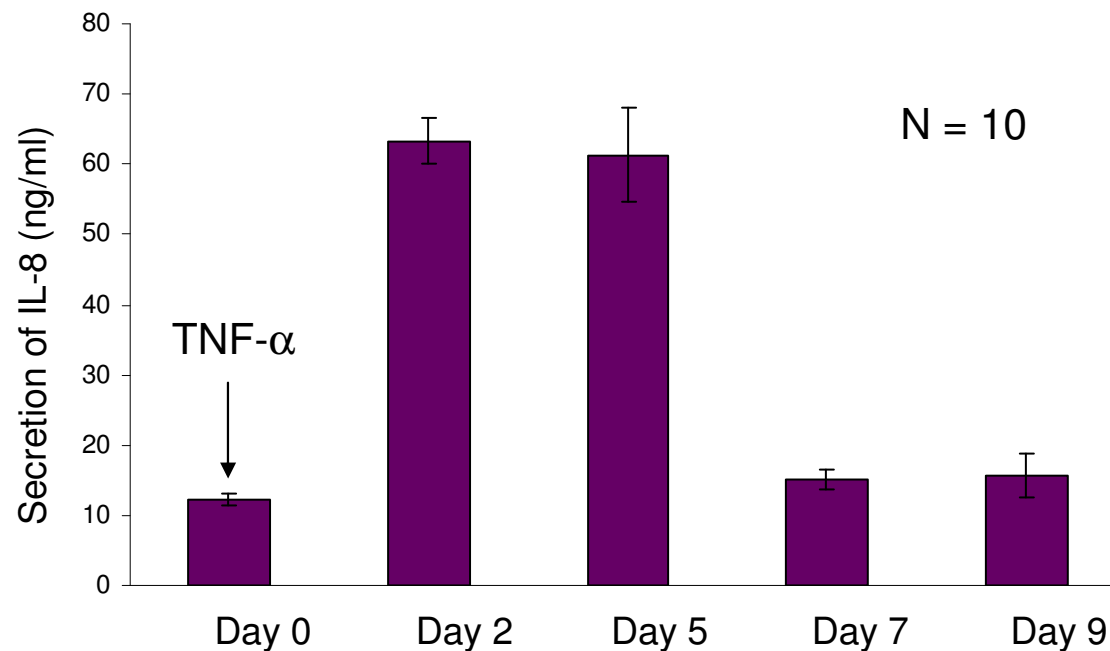
Basolateral exposure

- Ideal for gas, particles.
- Typical reaction with liquids :

- Ideal for liquids
- No inflammation induced by vehicle



Our primary *in vitro* epithelium responds to pro-inflammatory mediators (TNF- α) in a physiological manner

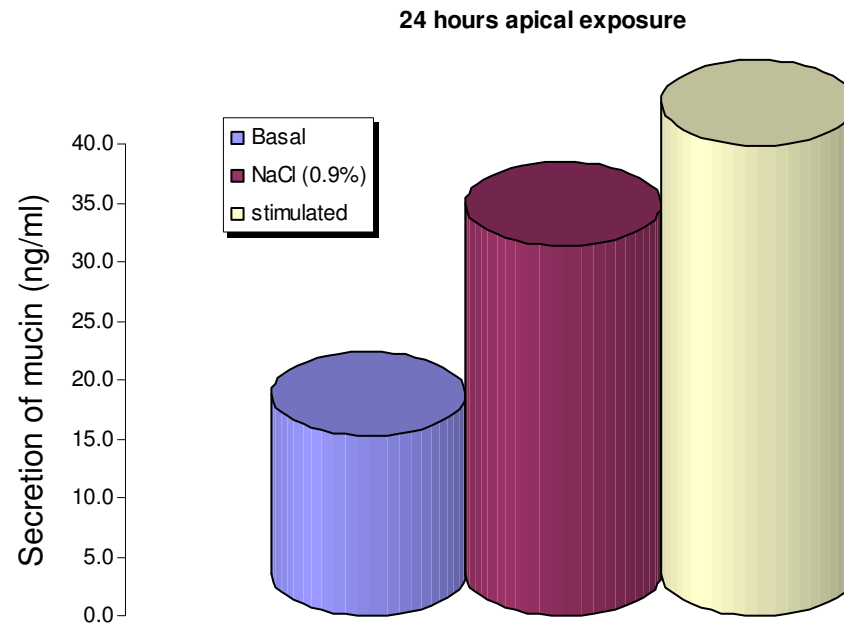


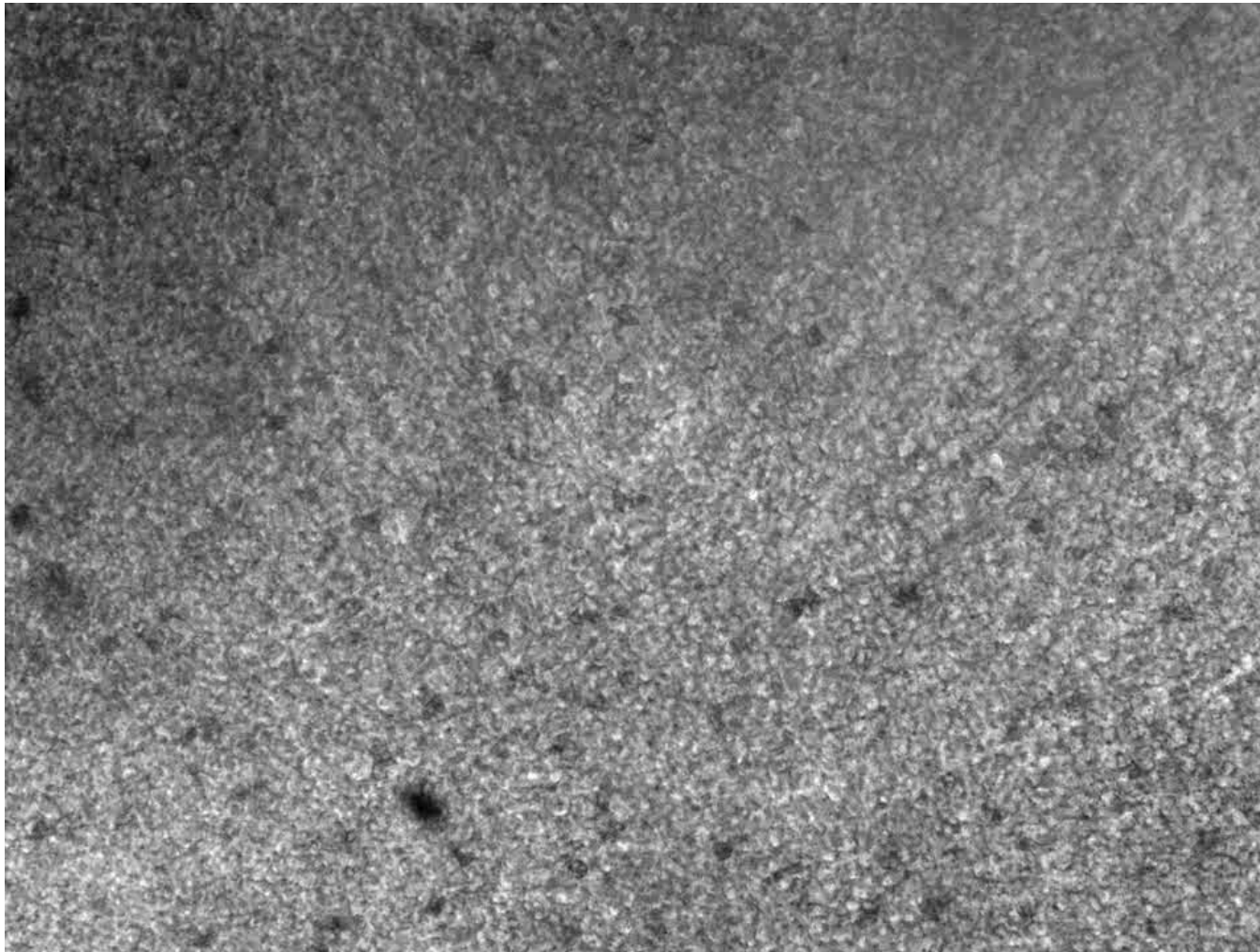
A time course of IL-8 secretion (an inflammatory marker) after 30 minutes of TNF- α stimulation on a 3 months old epithelium. Note that this same epithelium can be reused to simulate chronic inflammation.

Enzyme-linked lectin assay (ELLA)

The test is based on the affinity of lectins for mucins. It allows the quantitative measurement of the secreted mucins.

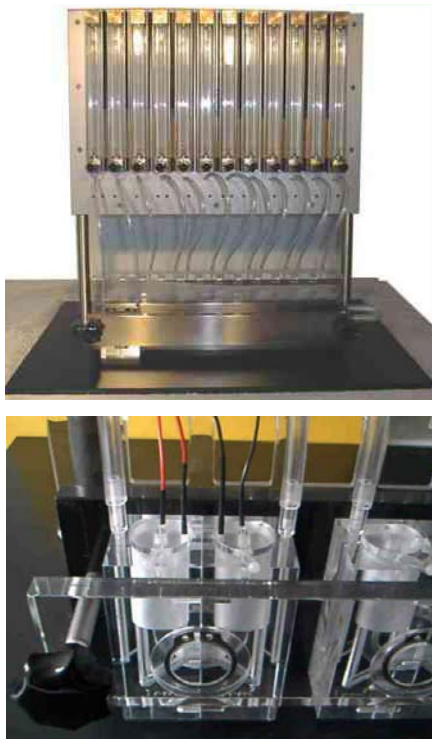
- Acute, long term or chronic exposition
- Positive control : ATP stimulation



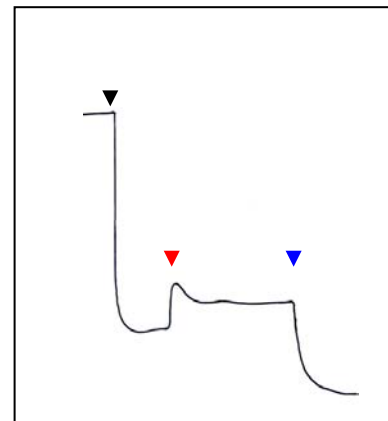


- Phase contrast or using fluorescent particles
- Direct measurement of particles velocity

The cells from our *in vitro* epithelium show typical electrophysiological responses to ion channel inhibitors and activators



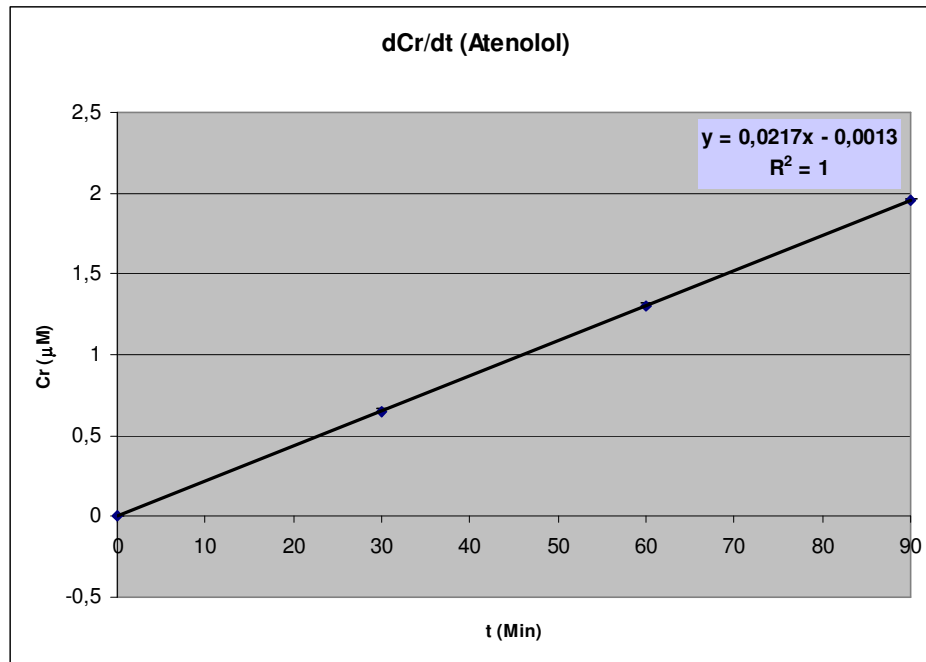
Current measurement in modified Ussing chamber



- ▼ Amiloride
- ▼ Isoproterenol
- ▼ Bumetanide

R($\Omega \cdot \text{cm}^2$) (resistance)
438 \pm 18 (N=203)

- Amiloride (sodium channel inhibitor) reduces the current.
- Isoproterenol, a CFTR activator (chloride channel) increases current.
- Bumetanide, a general channel blocker, abrogates the total channel current activities.



Molecule	P_{app} (cm/s)
Atenolol	55×10^{-6}
Propranolol	10×10^{-6}
Ibuprofen	157×10^{-6}
Insulin	2×10^{-6}

Time course of the rate of permeation of Atenolol from the apical to basal lateral side during a period of 90 min (triplicate).

- LC/MS detection
- High reproducibility

Acknowledgements



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Provider of human tissues reconstituted in vitro



- Ludovic Wiszniewski, Ph.D., CEO
- Song Huang, Ph.D., CSO
- Jean-Paul Derouette, Ph.D.
- Mireille Caul-Futy



Ligue suisse contre la vivisection



Fondation
Liechti

