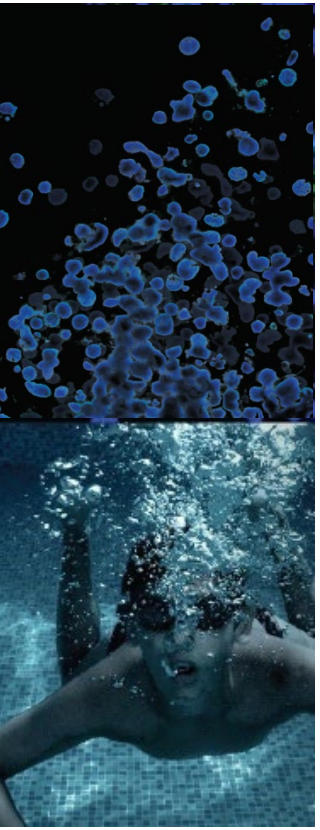
Primary and hTERT-immortalized Cells: Physiologically Relevant Cell Models for Toxicological Assays

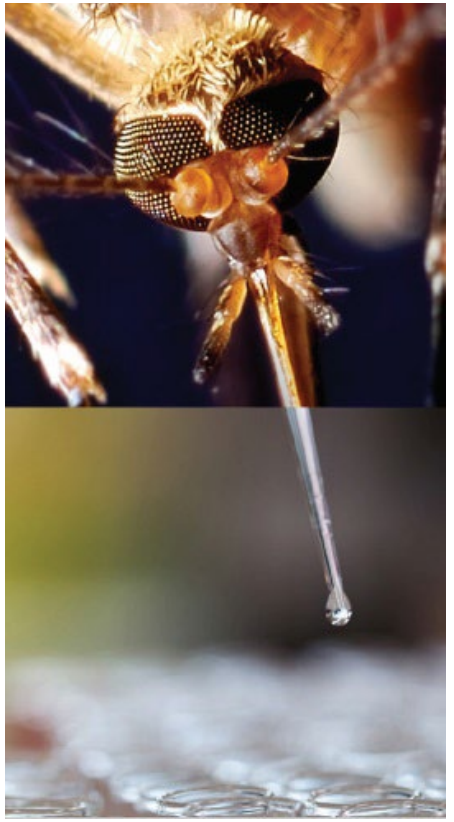


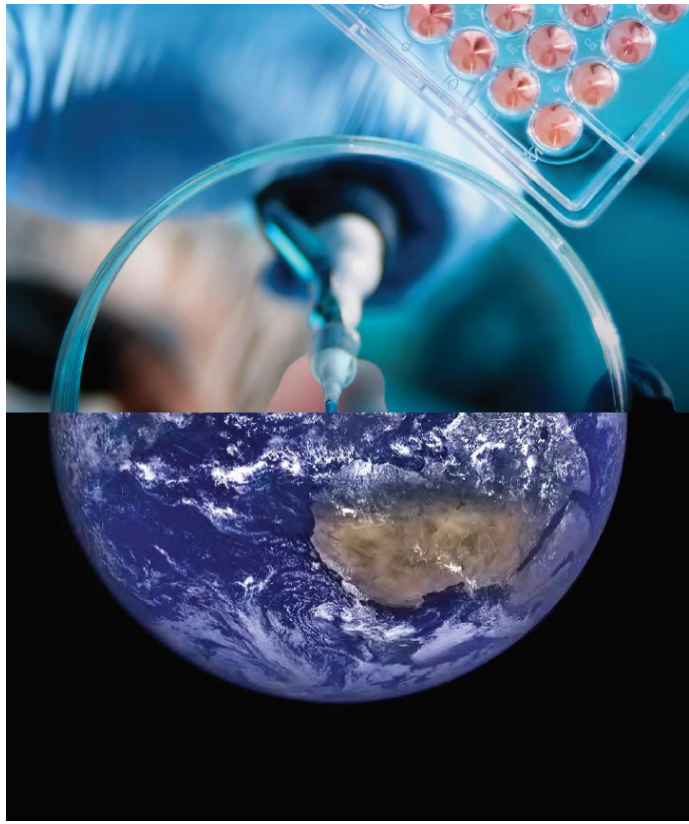
Carolina Lucchesi, PhD
BioNexus Foundation Principal Scientist, ATCC

Brian Shapiro, PhD
Market Segment Manager – Toxicology, ATCC

Sujoy Lahiri, PhD
Lead Scientist, ATCC

Credible Leads to Incredible™





About ATCC

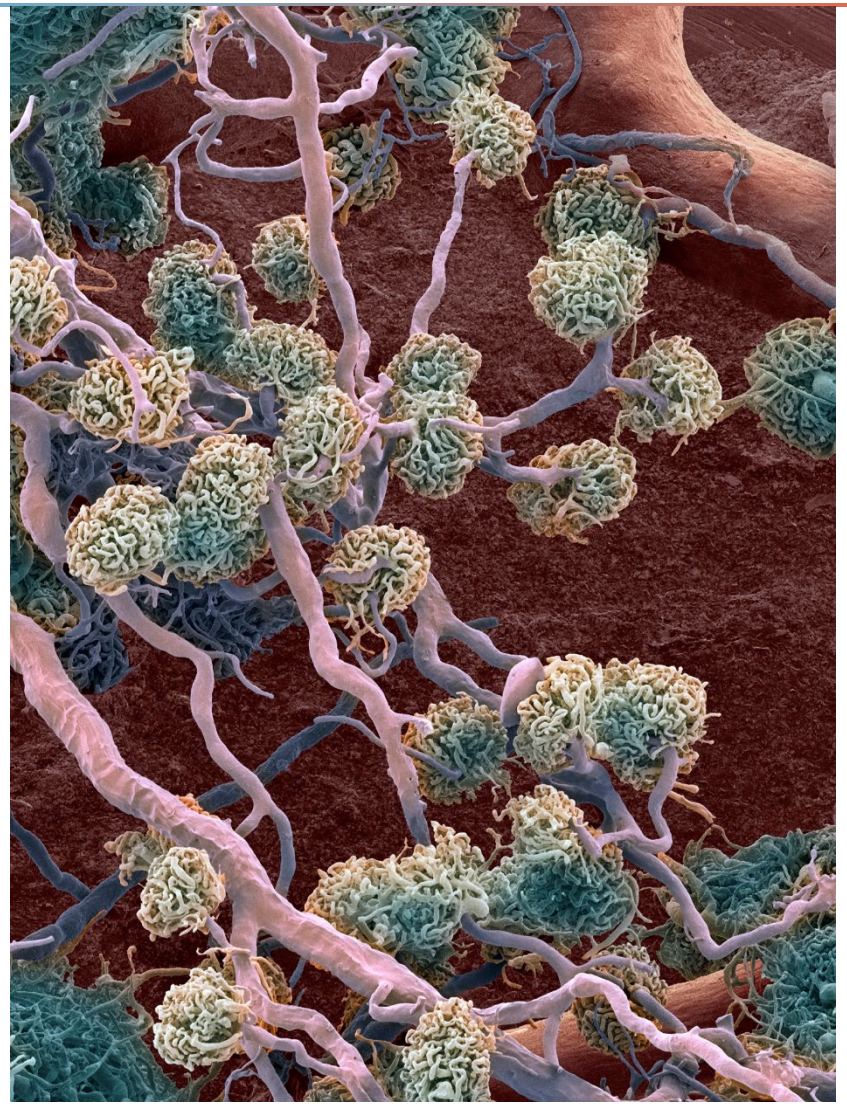
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring a growing portfolio of advanced 2-D and 3-D models
- cGMP biorepository

- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

ATCC®

Agenda

- ATCC mission and future direction
- ATCC toxicology portfolio
- Kidney models
- Airway models
- Dermal models



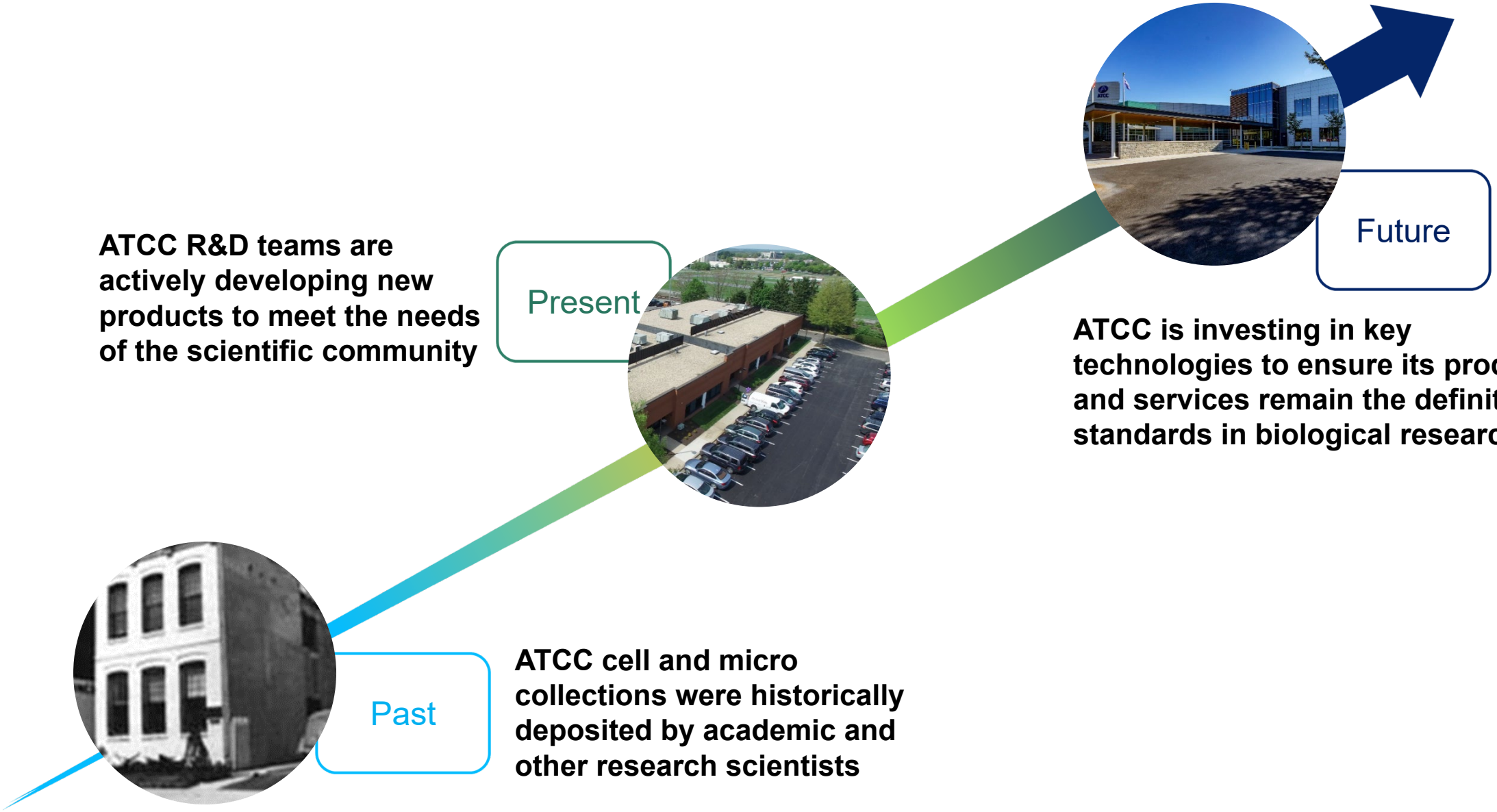
Mission and future direction

Carolina Lucchesi, PhD

BioNexus Foundation Principal Scientist, ATCC

Carolina Lucchesi is BioNexus Foundation Principal Scientist leading the Microphysiological Systems program at ATCC. Dr. Lucchesi received her PhD in Cellular and Molecular Biology from the University of Campinas in Brazil and has over 20 years of experience in Tissue Engineering and Organ-on-Chip technology. In her current role, Dr. Lucchesi leads the MPS program bringing new capabilities in the use of advanced 3D models and developing existing and new content to be applied in state-of-art technologies.

Modernization of the ATCC portfolio

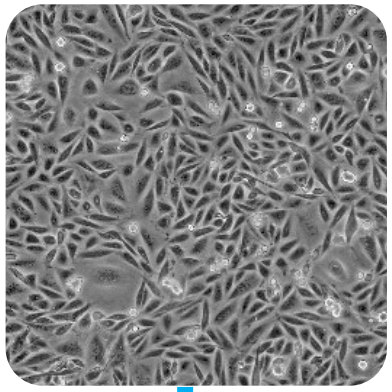


ATCC cell and micro collections were historically deposited by academic and other research scientists

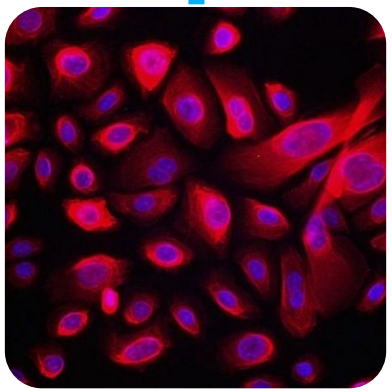
ATCC R&D teams are actively developing new products to meet the needs of the scientific community

ATCC is investing in key technologies to ensure its products and services remain the definitive standards in biological research

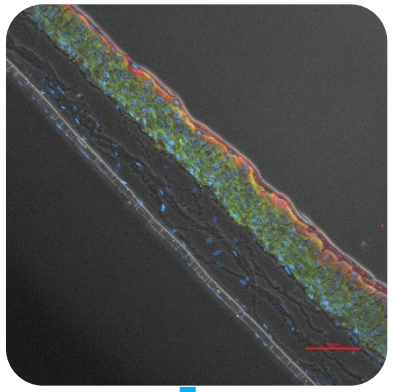
Evolution of in vitro cell models



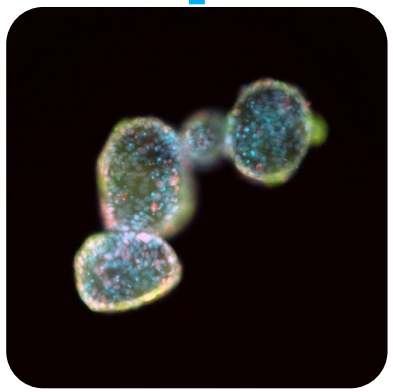
First generation
Continuous cell lines



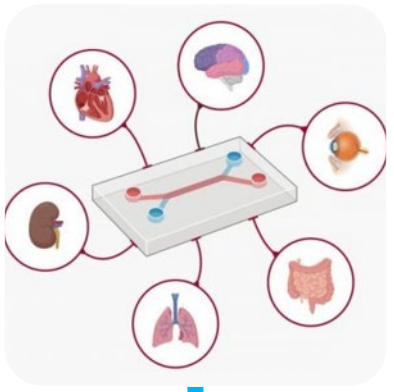
Second generation
Transfected cell lines
Reporter cell lines
Primary cells



Third generation
Immortalized primary cells
Gene-edited cell lines



Fourth generation
3D models
Organoids



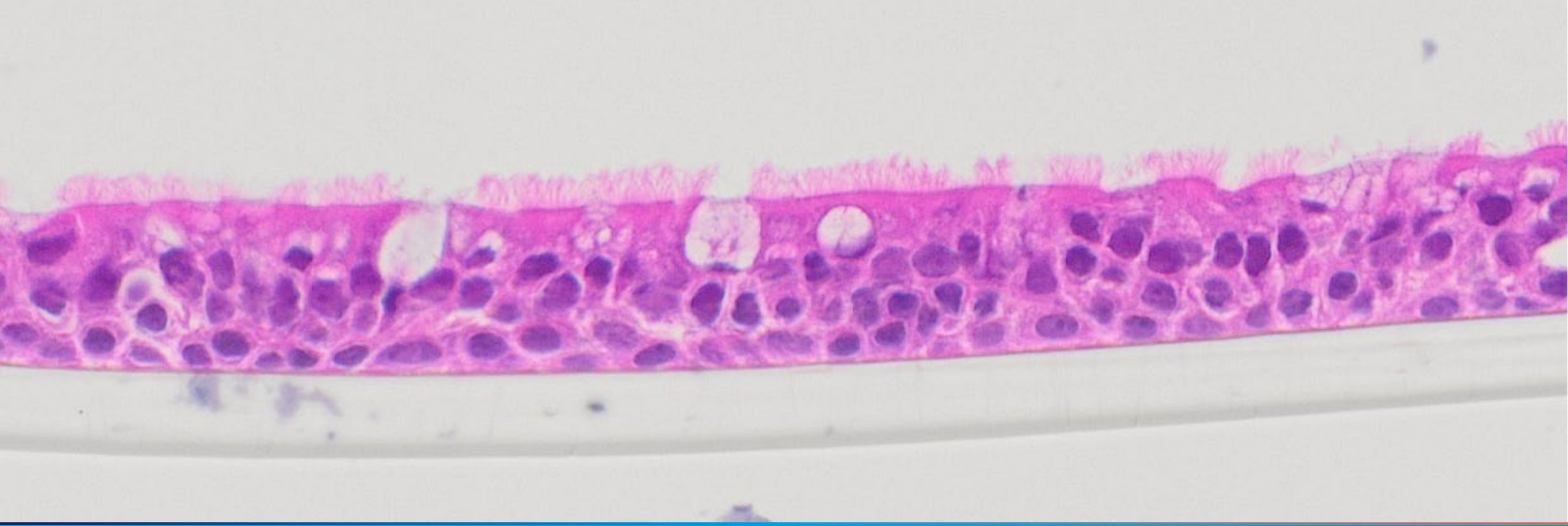
Fifth generation
Microphysiological systems
Organ-on-a-chip

ATCC toxicological models

Brian Shapiro, PhD

Market Segment Manager, Toxicology Segment, ATCC

Brian A Shapiro, PhD, works to drive revenue growth for ATCC's Cell Biology products through the development of relevant offerings, marketing strategies and execution of associated marketing plans. Previously, he worked at Virginia Commonwealth University, where he investigated the role of pre-mRNA splicing in the multi-drug resistance of lung cancer. Dr. Shapiro attended the Medical College of Georgia, where his research focused on adrenal physiology as well as diseases of the epidermis.



ATCC Toxicological Models

Comparison of various cell models

	Continuous (cancer) cell lines	Stem cells (eg, iPSCs, MSCs)	Primary cells	hTERT-immortalized primary cells
Mimic *in vivo* characteristics	Suboptimal	Optimal	Optimal	Optimal
Proliferative capacity	Optimal	Depends on type/conditions	Suboptimal	Optimal
Experimental reproducibility	Optimal	Optimal	Suboptimal	Optimal
Predictability in toxicological studies	Suboptimal	Upon differentiation	Optimal	Optimal
Genomic stability	Aneuploid	Diploid	Diploid	Diploid/near diploid
Supply	Optimal	Optimal	Suboptimal	Optimal
Cost	Optimal	Suboptimal	Suboptimal	Optimal
Ease of use	Easy	Can be challenging	Can be challenging	Easy

Primary: Ideal when donor diversity is needed
Immortalized: Ideal for screening or when a consistent source is needed

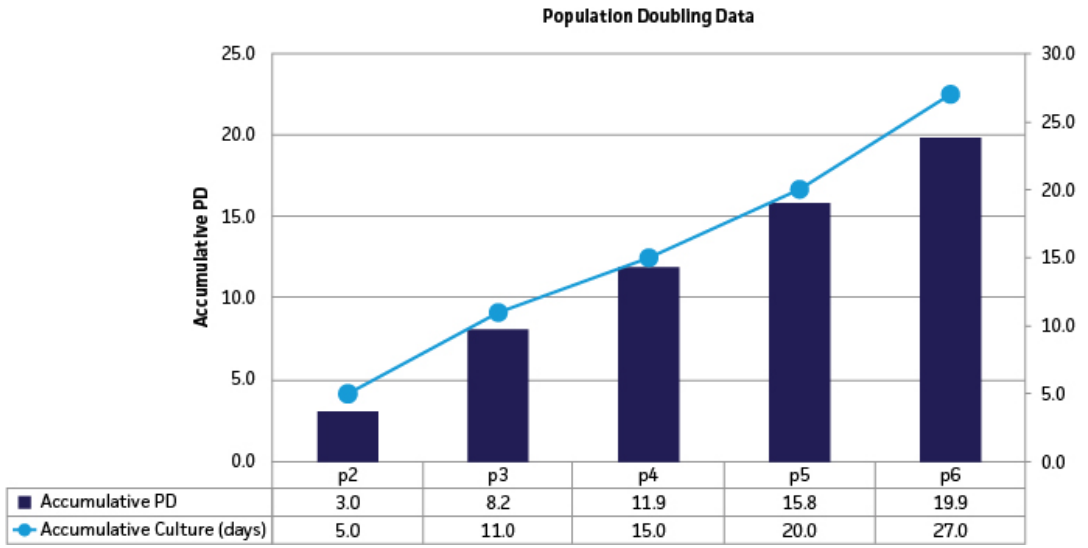
ATCC

Primary cells – Key characteristics

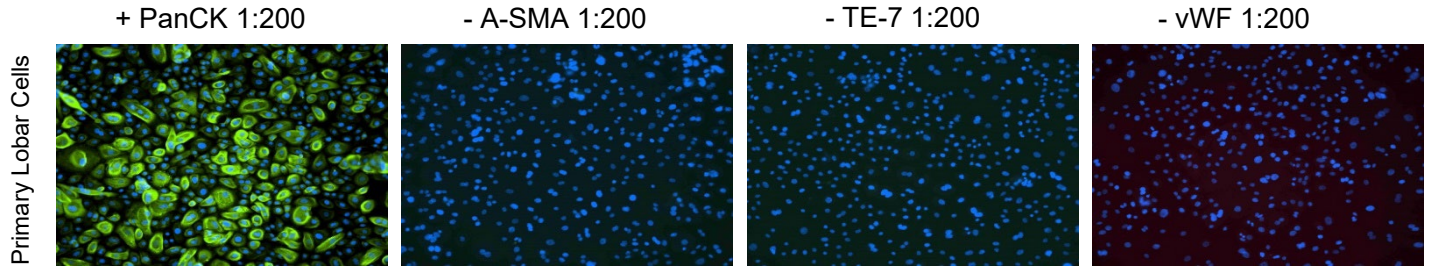
Isolated directly from primary donor tissue, human primary cells more closely mimic the physiological state of cells in vivo and generate more relevant data representing living systems.

- Growth
  - Supplied at P0 or P2
  - At least > 10 (usually 15) doublings guaranteed
- Characterization
  - Bacterial, fungal, mycoplasmal, viral testing
  - Specific positive and negative markers confirmed via ICC or FACS
- Toxicology responses
  - Analogous to in vivo

Bladder Epithelial Cells(A/T/N)

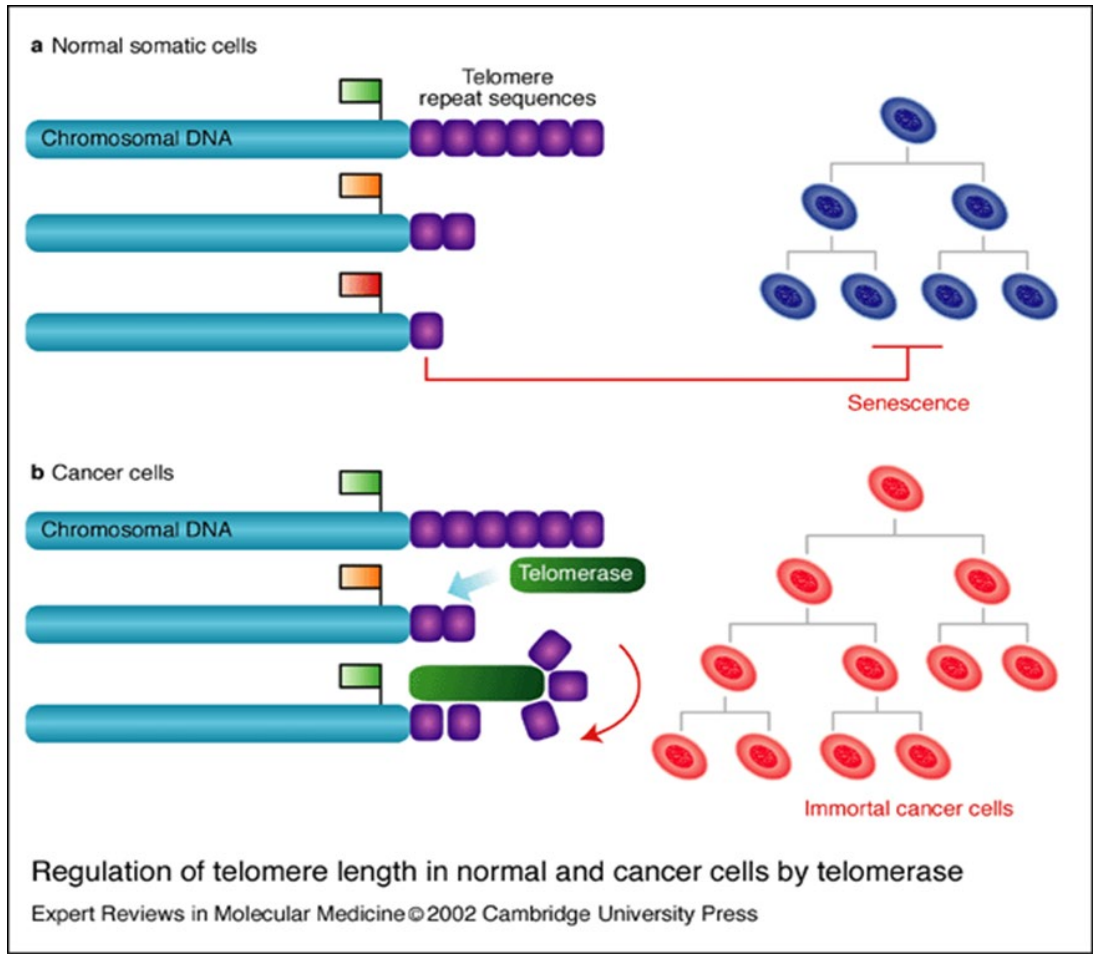


	p2	p3	p4	p5	p6
Accumulative PD	3.0	8.2	11.9	15.8	19.9
Accumulative Culture (days)	5.0	11.0	15.0	20.0	27.0



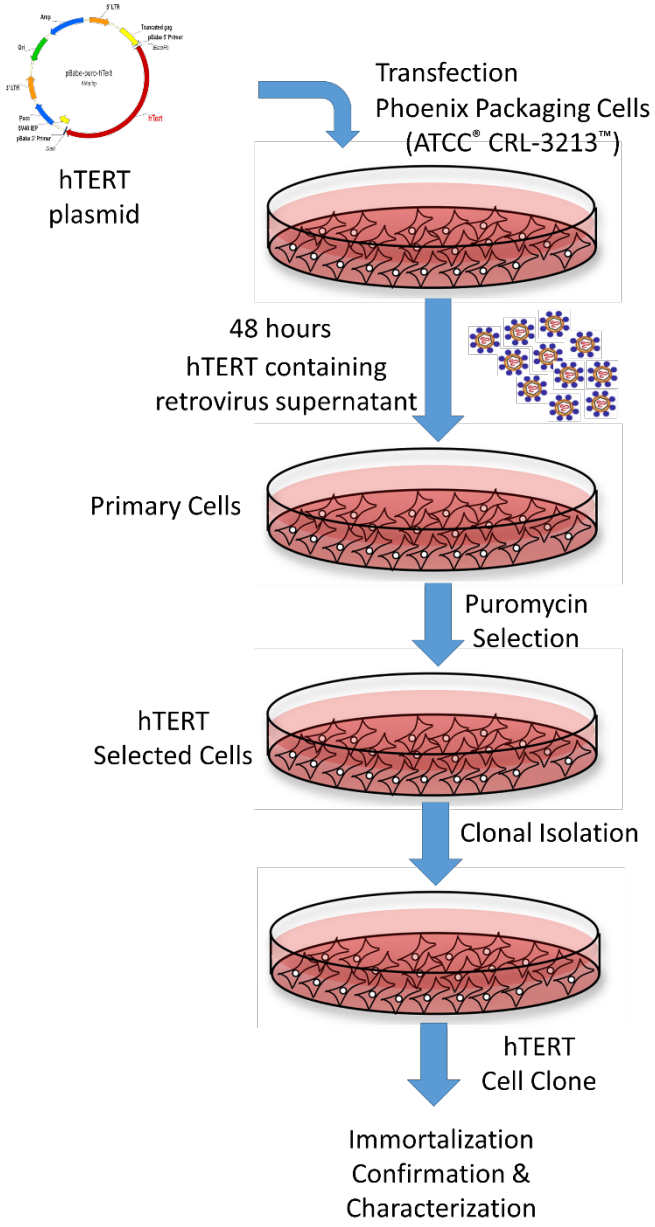
Lobar Epithelial Cells are positive for PanCK and negative for TE-7, α-SMA, and vWF

hTERT-immortalization technology



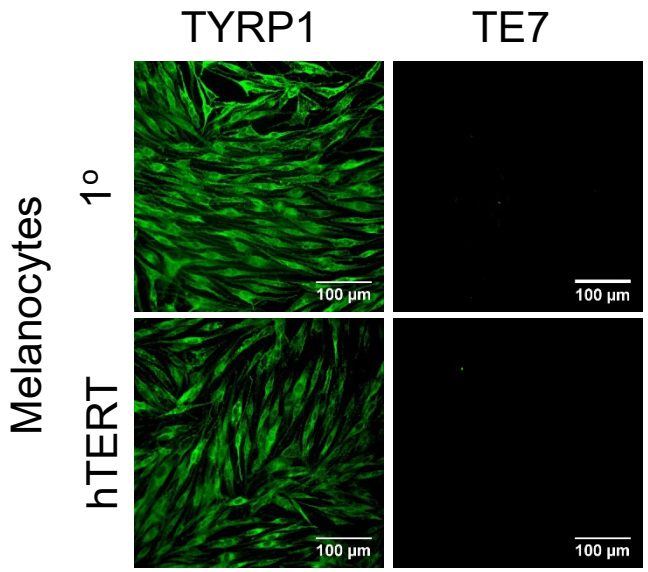
Regulation of telomere length in normal and cancer cells by telomerase

Expert Reviews in Molecular Medicine ©2002 Cambridge University Press

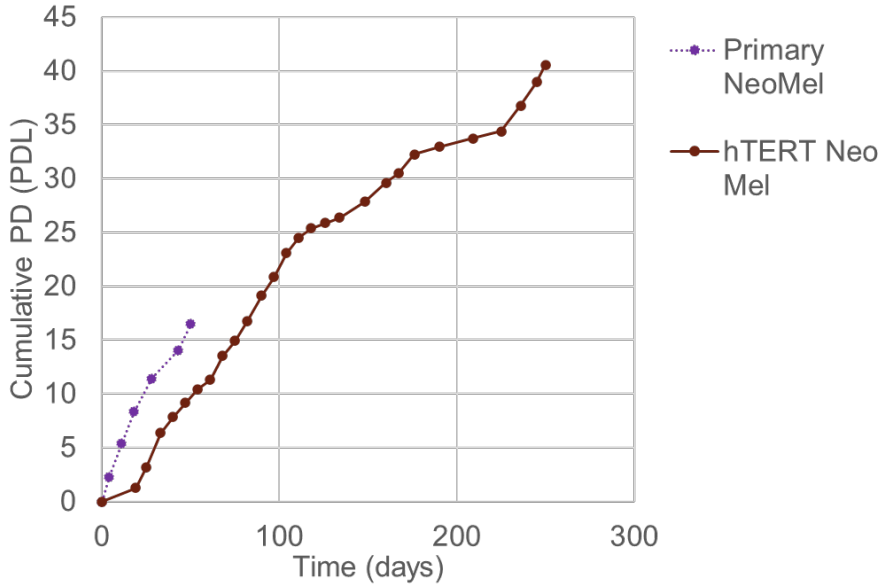


hTERT-immortalized cells – Key characteristics

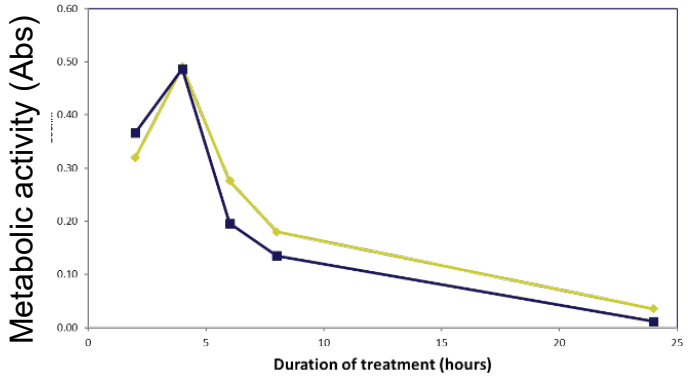
- Growth
  - Cells retain replicative capacity ("immortalized")
  - Population doubling rate is comparable to primary cells
- Characterization
  - Morphology and marker expression similar to primary cells
  - Sterility testing
  - STR profile
- Toxicology responses
  - Within expected range
  - Analogous to primary cells



Growth of ATCC® CRL-4064™ Neonatal Dermal Melanocytes



Metabolic reduction by 3D organotypic skin culture in Triton-X



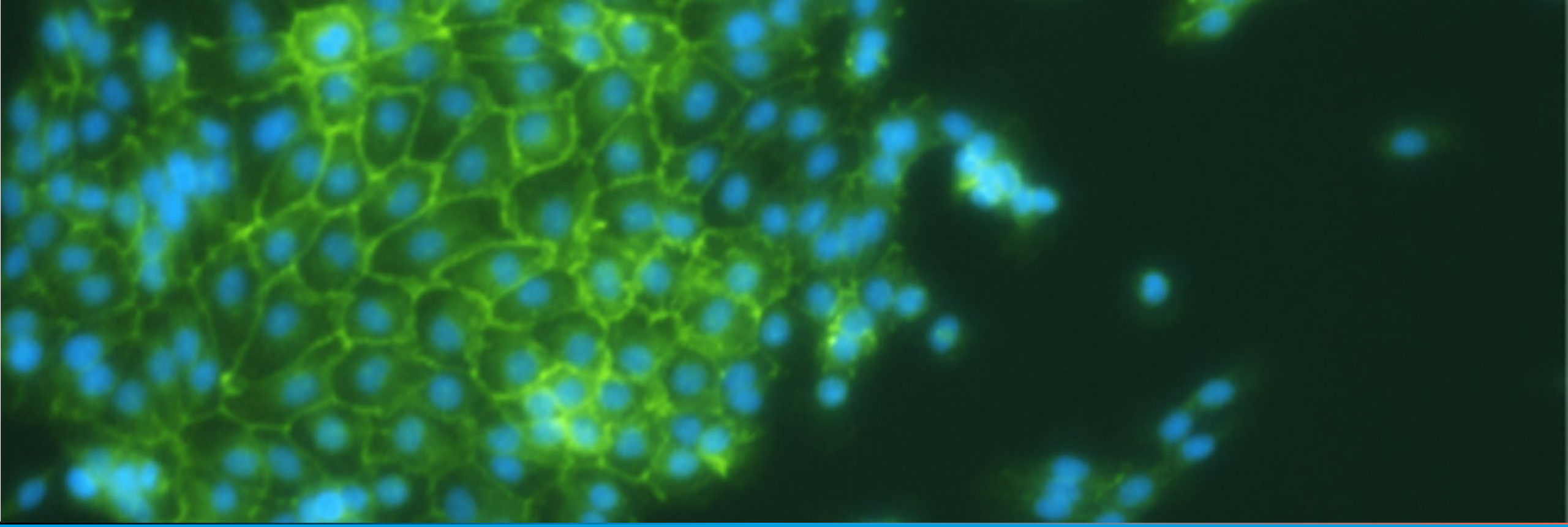
Time (hrs)

Kidney, airway, and dermal models and functionality

Sujoy Lahiri, PhD

Lead Scientist, ATCC

Sujoy Lahiri, PhD, is an R&D scientist in ATCC. He leads the primary cell division, working on advanced cellular models using primary cells as well as expansion of ATCC's immortalized primary cell portfolio. Dr. Lahiri has extensive knowledge in the field of toxicology and drug metabolism. Previously, Dr. Lahiri worked at National Institutes of Health, where his work focused on lipid biochemistry. Dr. Lahiri received his PhD from the Weizmann Institute of Science, where he studied sphingolipid biochemistry and metabolism.



Kidney Models and Functionality

ATCC

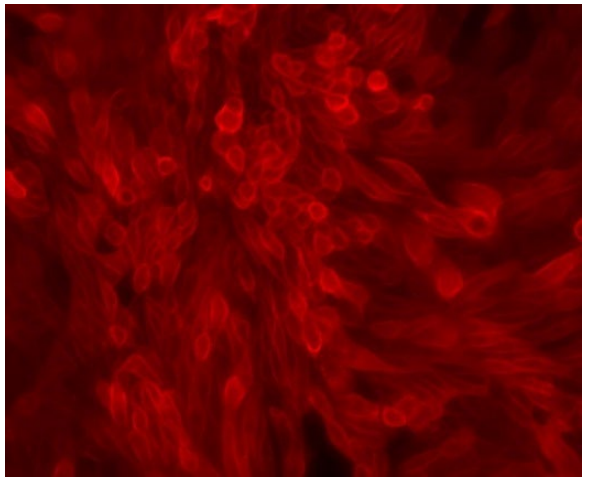
Kidney models

Renal proximal tubule epithelial cells

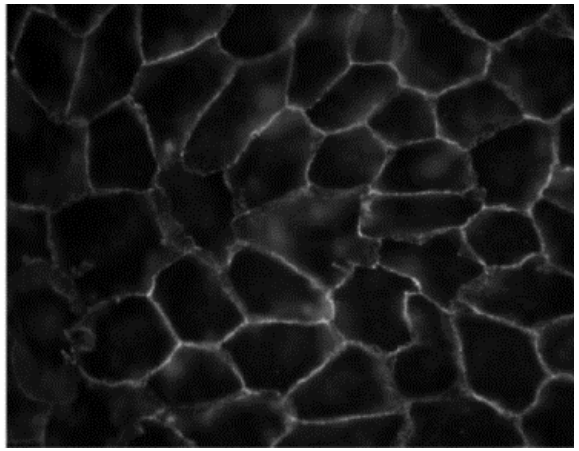
- Primary renal proximal tubule epithelial cells
- hTERT-RPTEC – immortalized renal proximal tubule epithelial cells

- Key characteristics:
  - Uniform expression of E-cadherin and CD13 (aminopeptidase N)
  - Formation of dome-like structures
  - Stabilized transepithelial electrical resistance (TEER)

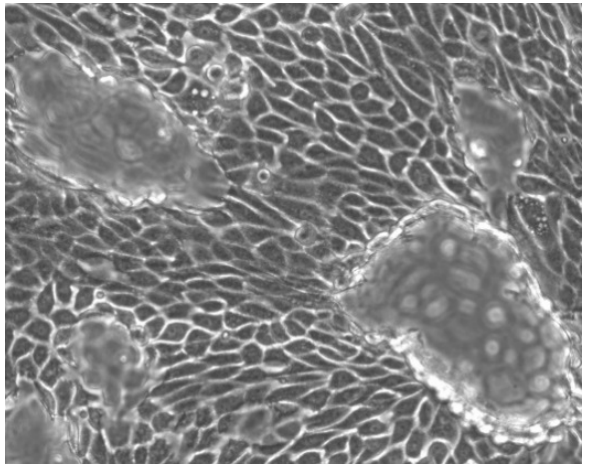
RPTEC/TERT1: CD13

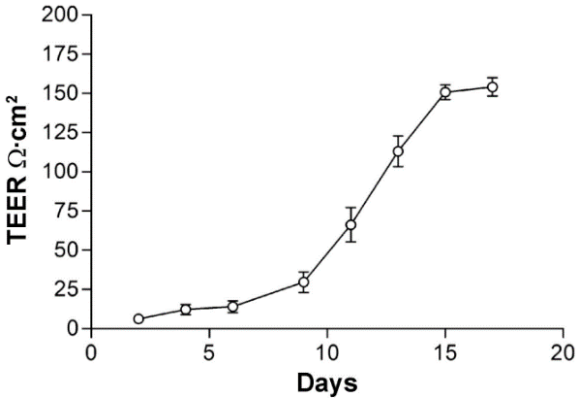


RPTEC/TERT1: E-cadherin

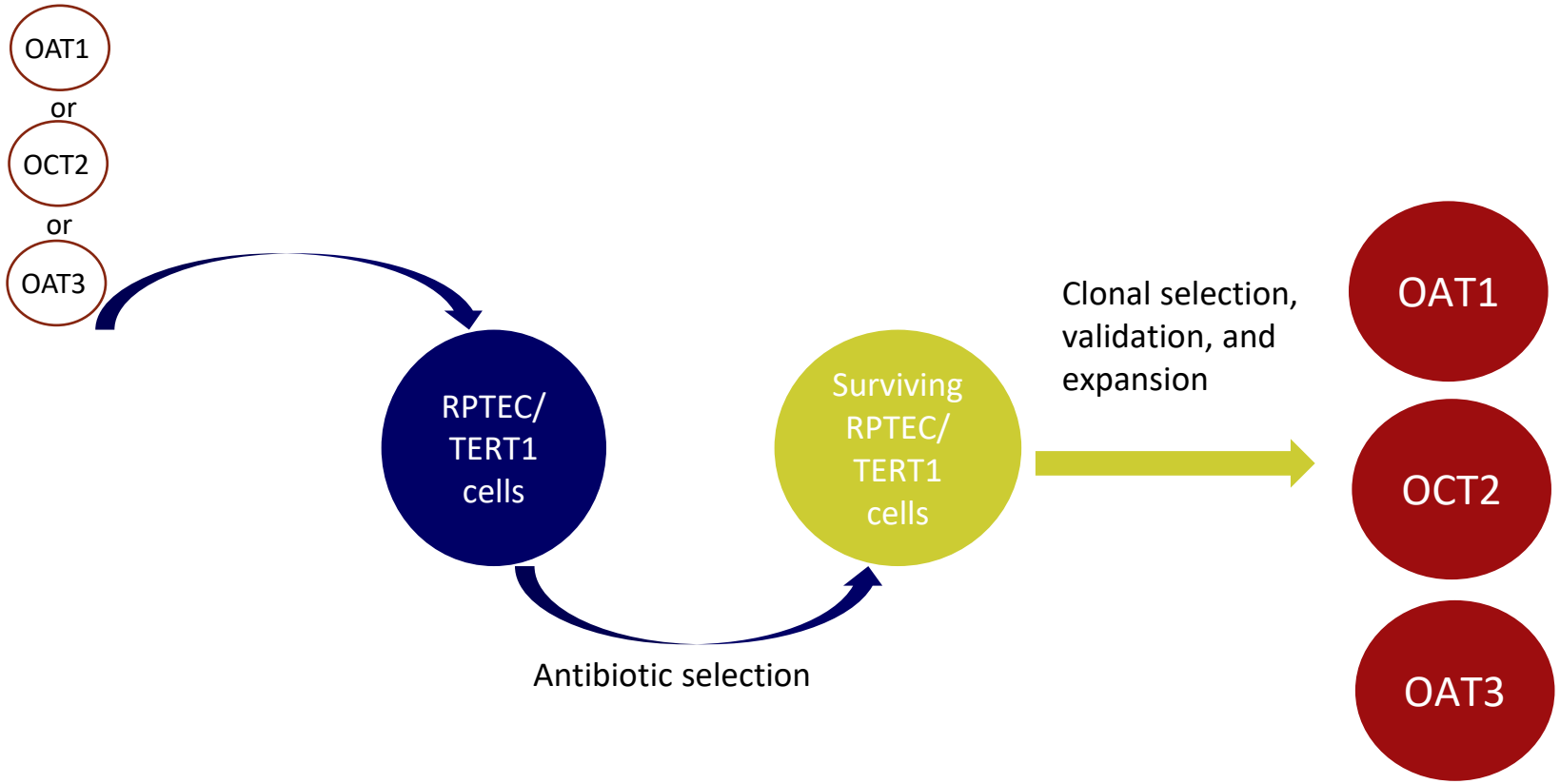


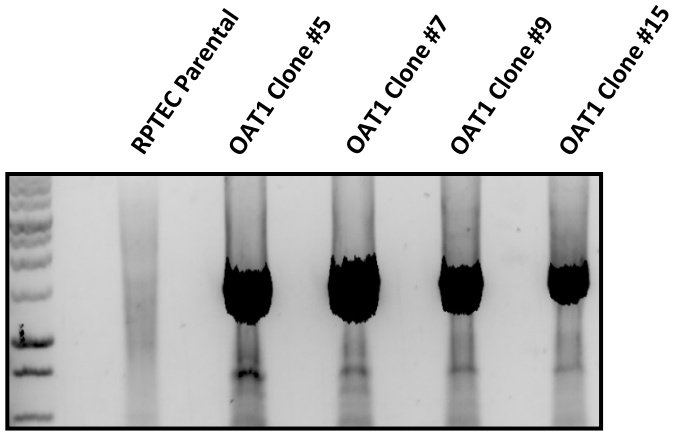
Dome formation

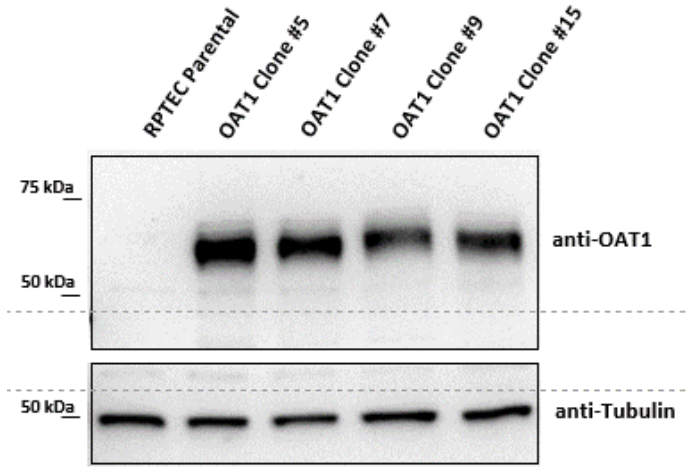




Enhanced kidney cellular models







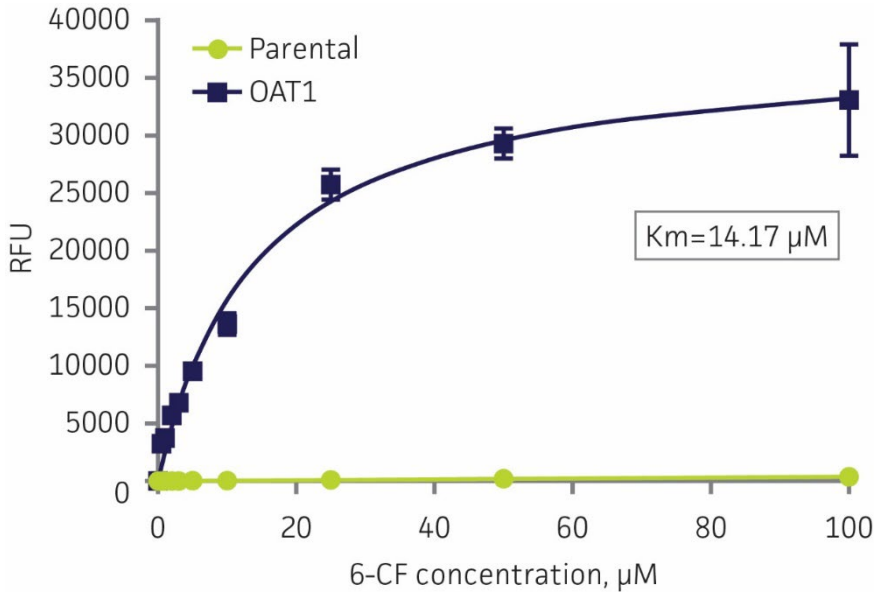
- RPTEC/TERT1
- RPTEC/TERT1 OAT1
- RPTEC/TERT1 OCT2
- RPTEC/TERT1 OAT3

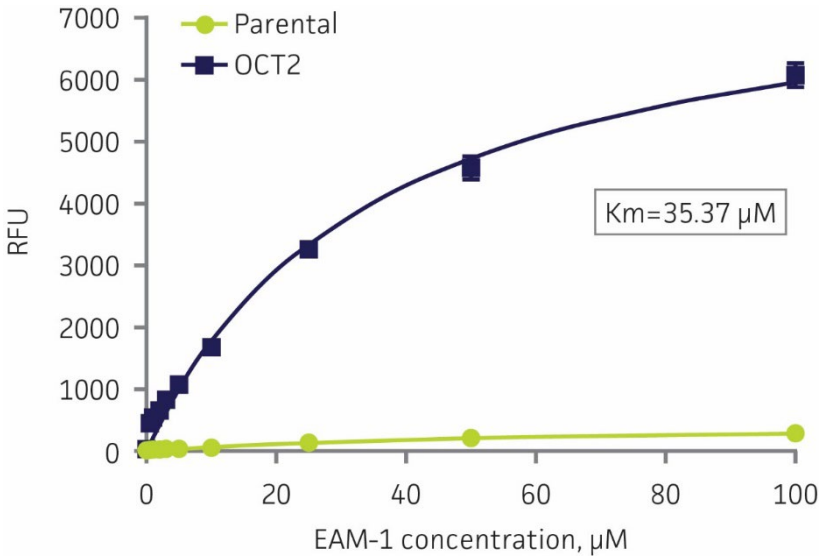
Characterized by
RT-PCR, WB, sequencing
(copy number verified)

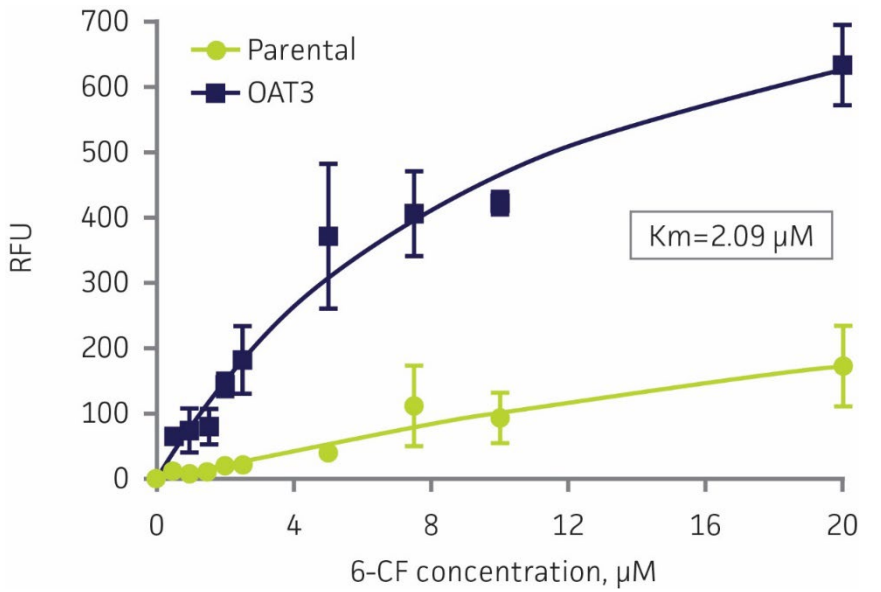
Functionality – Drug uptake assay

UPTAKE ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours
- Increasing concentration of 6-CF or EAM1 were added and incubated for 20 minutes at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured





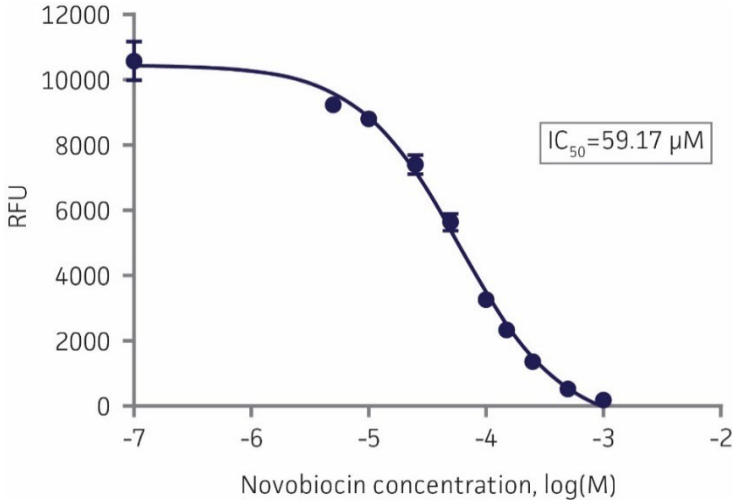


Functionality – Drug uptake inhibition assay

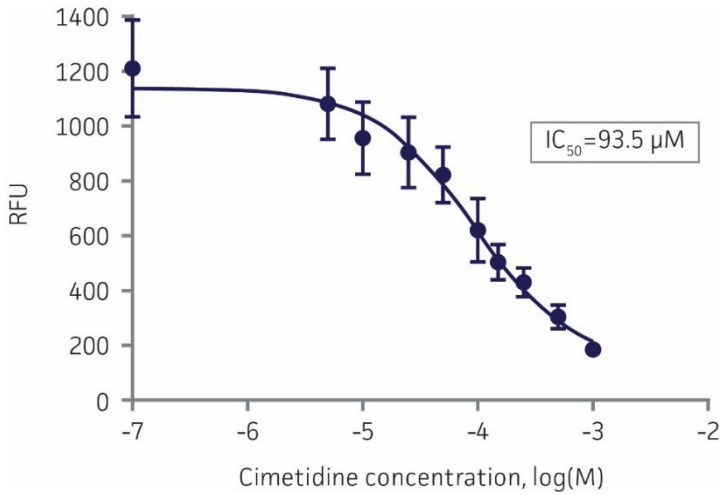
UPTAKE INHIBITION ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours
- Increasing concentration of inhibitors were added together with constant concentrations of the uptake substrate and incubated for 20 mins at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured

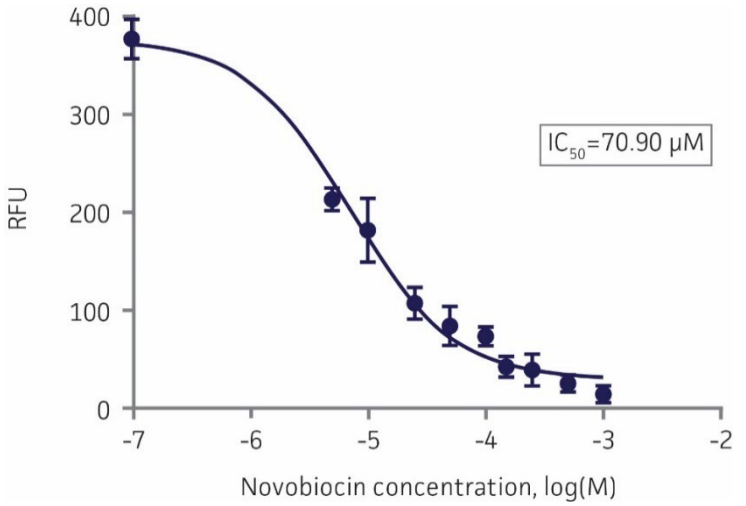
6-CF uptake inhibition in OAT-1 expressing RPTEC



EAM-1 uptake inhibition in OCT-2 expressing RPTEC



6-CF uptake inhibition in OAT-3 expressing RPTEC



RPTEC-OCT2 – Drug-drug Interactions (DDI) application

The problem

- A multitude of disease and therapy related factors drive the frequent development of renal disorders in cancer patients
- Many cancer patients have comorbidities such as urinary tract infections, tuberculosis, and diabetes
- Commonly prescribed medications such as levofloxacin (TEA) or metformin can interact with chemotherapeutics
- These common medications can block the renal uptake of the candidate via organic cation SLC transporters

The solution: Use SLC transporter cells to identify DDI

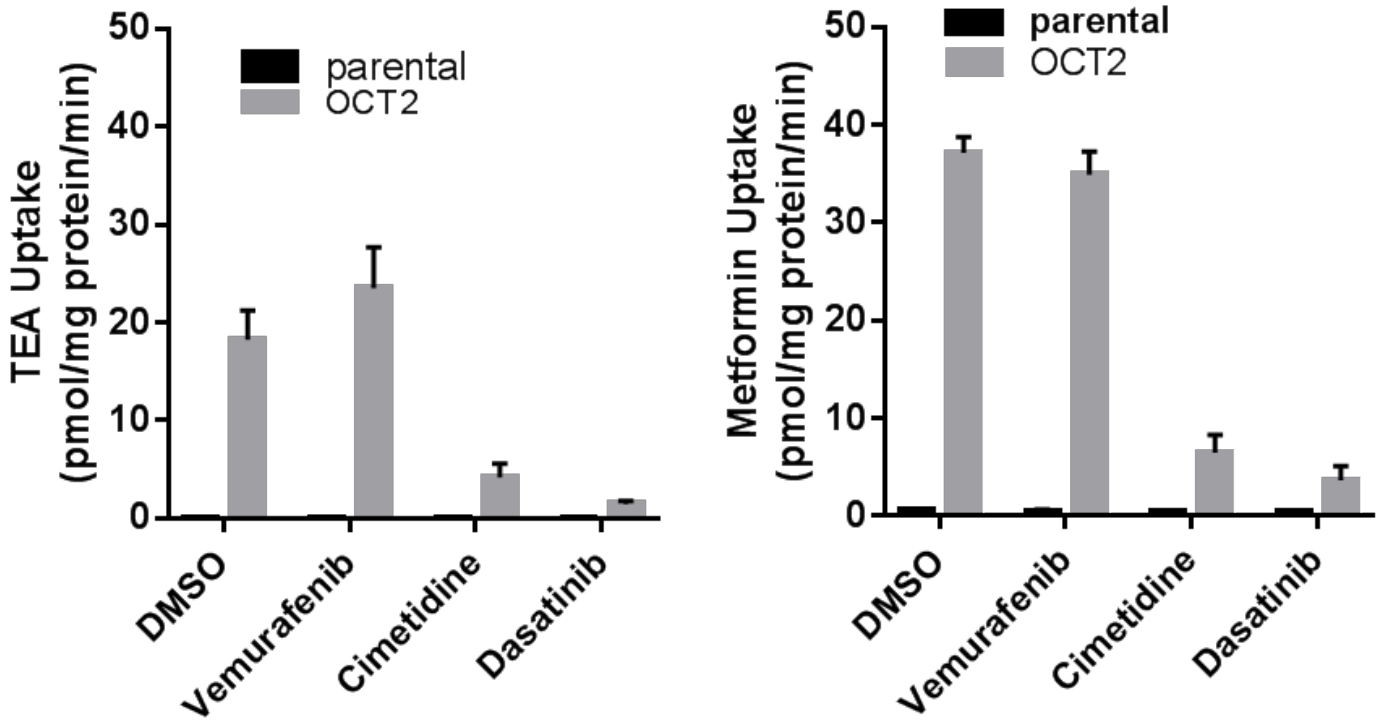
- Incubate candidate drugs with radiolabeled known SLC substrate drugs in RPTEC-OCT2 cultures, monitor uptake
- If uptake of radiolabeled compounds is inhibited, then DDI is indicated

Uptake inhibition assay protocol

- Aspirate growth media and wash once with warm 1X PBS; remove PBS and add 250 µL of cold inhibitors (prepared serum free DMEM, 0.5 µM) and incubate for 15 minutes
- Remove inhibitors and add 250 µL of radio-labeled TEA or metformin (prepared serum free DMEM, 4.5 µM) and incubate for 15 minutes
- Remove drug and wash 3 times with cold PBS; lyse the cells and count

RPTEC-OCT2 – Drug-drug Interactions (DDI) application

Drug-Drug Interactions



Data kindly provided by:

Kevin Huang, *Graduate Research Associate,* Ohio State University, College of Pharmacy

Alice Gibson, Ph.D., *Senior Research Specialist,* Ohio State University, College of Pharmacy

RPTEC-OAT1 – Nephron toxicity application

The problem

- Many disease and therapy related factors drive the frequent development of renal disorders in cancer patients
- Targeted therapeutics can cause renal dysfunction through on and off-target mechanisms
- Small-molecule inhibitors approved for the treatment of cancers can trigger tubular damage and acute kidney injury (AKI)

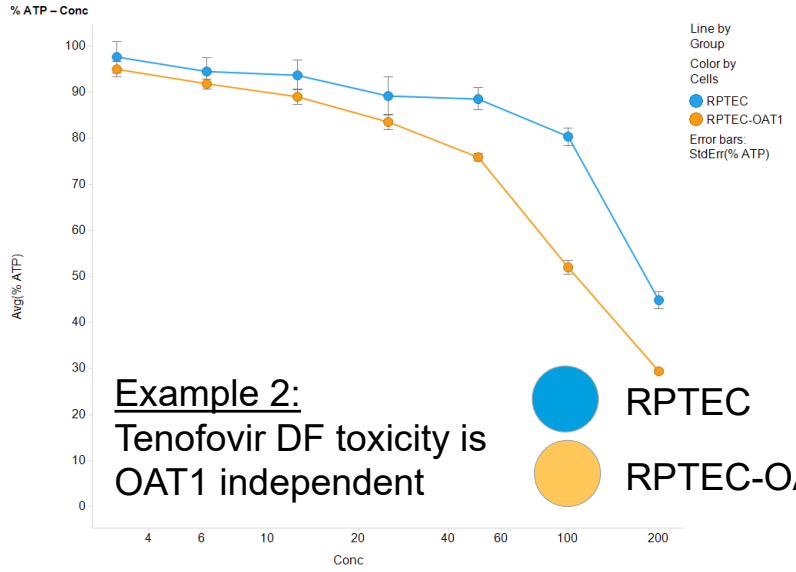
The solution: Use SLC transporter cells to identify nephron toxicity

- Incubate candidate in RPTEC-OAT1 or parental RPTEC cells, monitor cytoxicity
- If the kill curves are similar, toxicity is OAT1 independent
- If the kill curves are different, toxicity is OAT1 dependent

Cell viability assay protocol

- About 35000 cells were seeded per well in triplicate in a 96-well plate and incubated overnight
- Cells were incubated with a series of compounds at various concentrations for 3 days
- Cell viability was determined using a cell viability assay per manufacturer's instructions

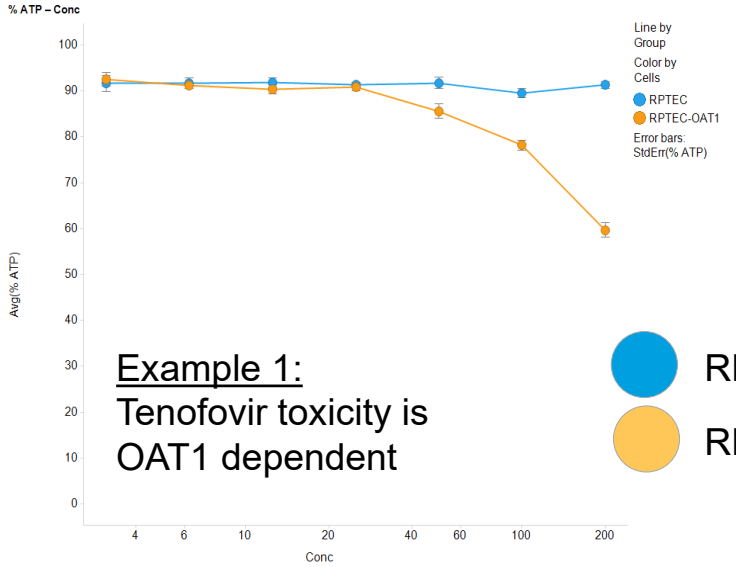
Nephron toxicity application



Example 2:
Tenofovir DF toxicity is OAT1 independent

RPTEC

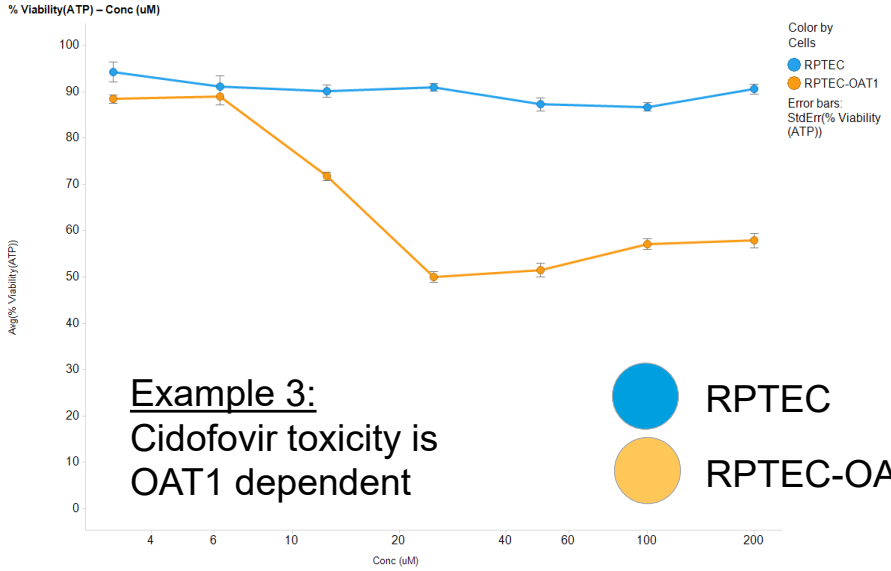
RPTEC-OAT1



Example 1:
Tenofovir toxicity is OAT1 dependent

RPTEC

RPTEC-OAT1



Example 3:
Cidofovir toxicity is OAT1 dependent

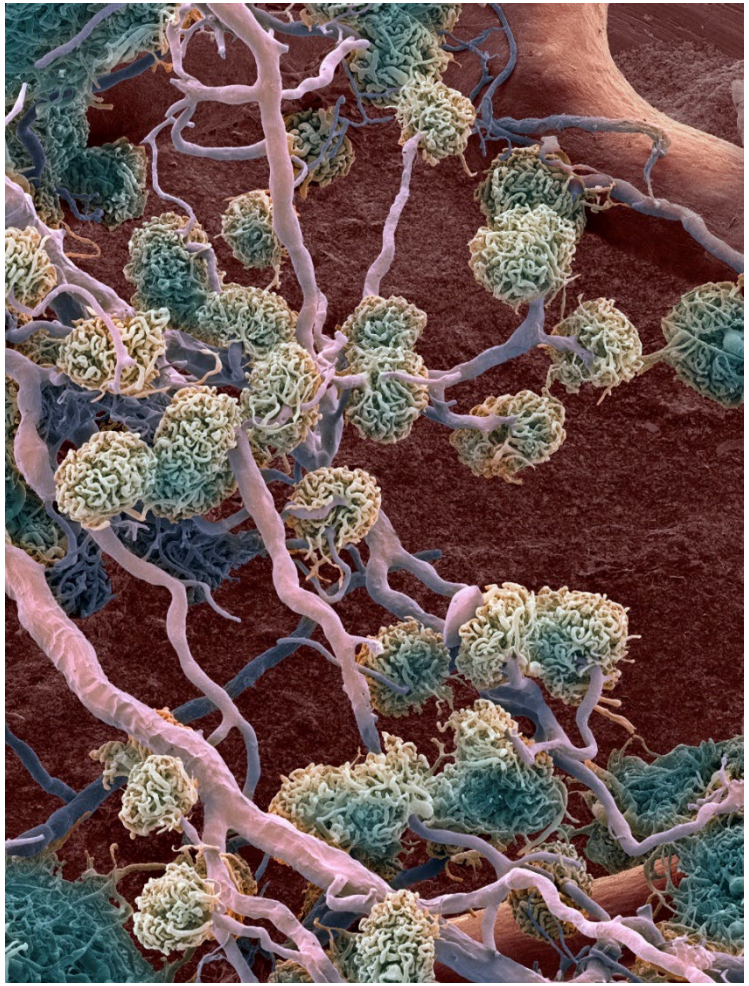
RPTEC

RPTEC-OAT1

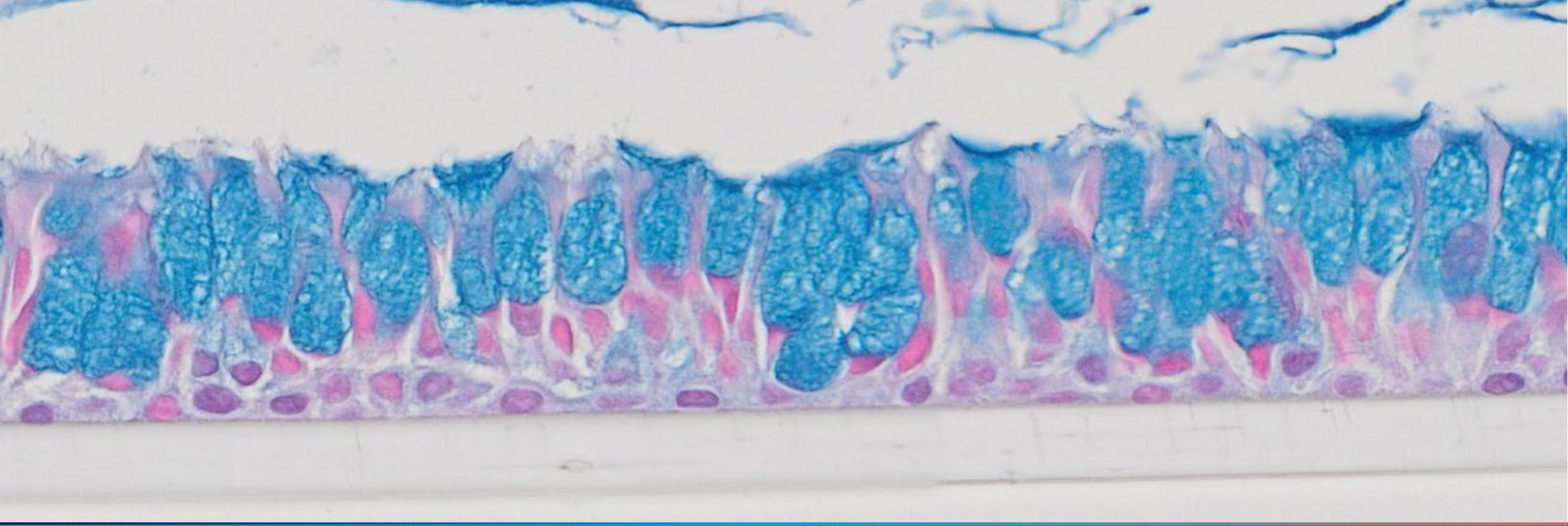
Data kindly provided by: Merck & Co., Inc.

Summary of kidney models

- ATCC primary and hTERT-immortalized RPTEC display the many key in vivo characteristics
- We enhanced h-TERT-immortalized RPTEC with organic anion/cation transporter proteins.
- Our internal data indicates:
  - Uptake of specific fluorescent substrates is enhanced vs parental cells
  - Substrate drug uptake is reduced by known OAT1/OAT3 and OCT2 inhibitors
- Data from external collaborators indicates:
  - RPTEC-OCT2 has drug-drug Interactions (DDI) applications
  - RPTEC-OAT1 has nephron toxicity applications



ATCC



Airway Models and Functionality

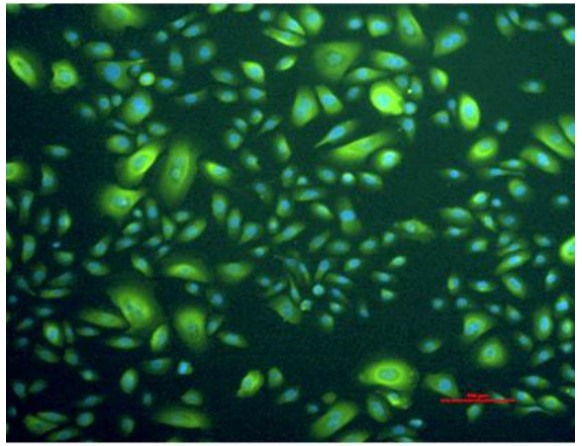
ATCC®

ATCC products for airway models

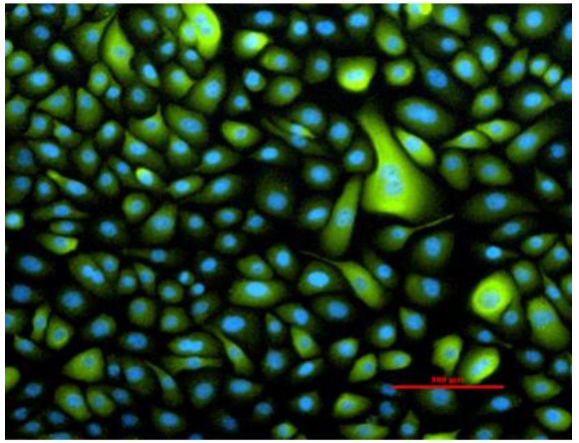
Cell type	Primary	hTERT-immortalized	Normal/Disease
Epithelial	• Bronchial/tracheal • Small Airway • Lobar	• Bronchial epithelial • Small airway	• Normal • COPD • Fibrosis • Asthma • Cystic fibrosis
Fibroblast	• Lung	• Lung	• Normal • COPD • Fibrosis • Asthma
Smooth muscle	• Bronchial/tracheal • Lung	• N/A	• Normal

CCSP + DAPI

Primary Bronchial/Tracheal Epithelial Cells



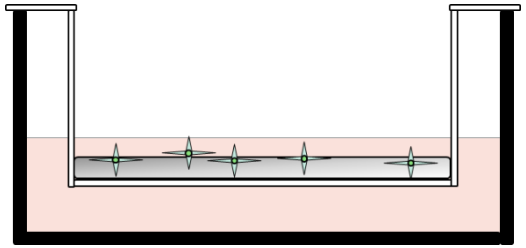
HSAEC1-KT



Overview of airway model fabrication

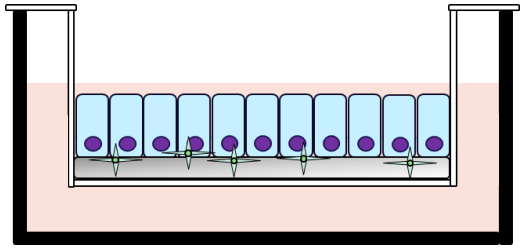
Coat trans-well insert
with collagen (100 µL)

24 Hr



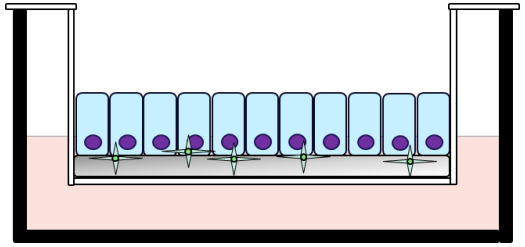
Add fibroblasts to apical layer
(20,000 cells)

24 Hr



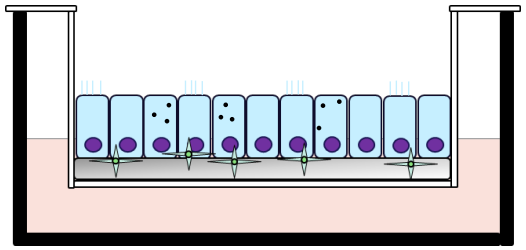
Add epithelial cells to apical layer
(100,000 cells)

48-72 Hr

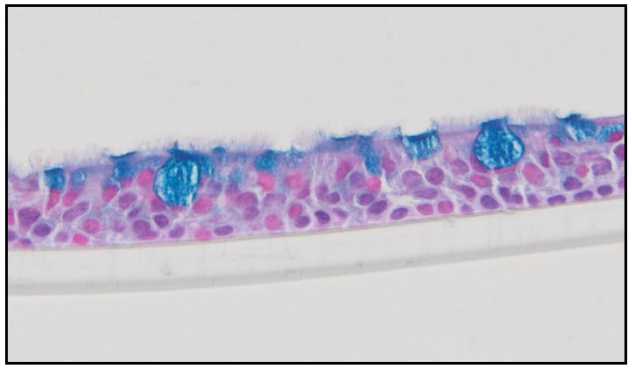


Once confluent, remove apical
media to begin ALI culturing

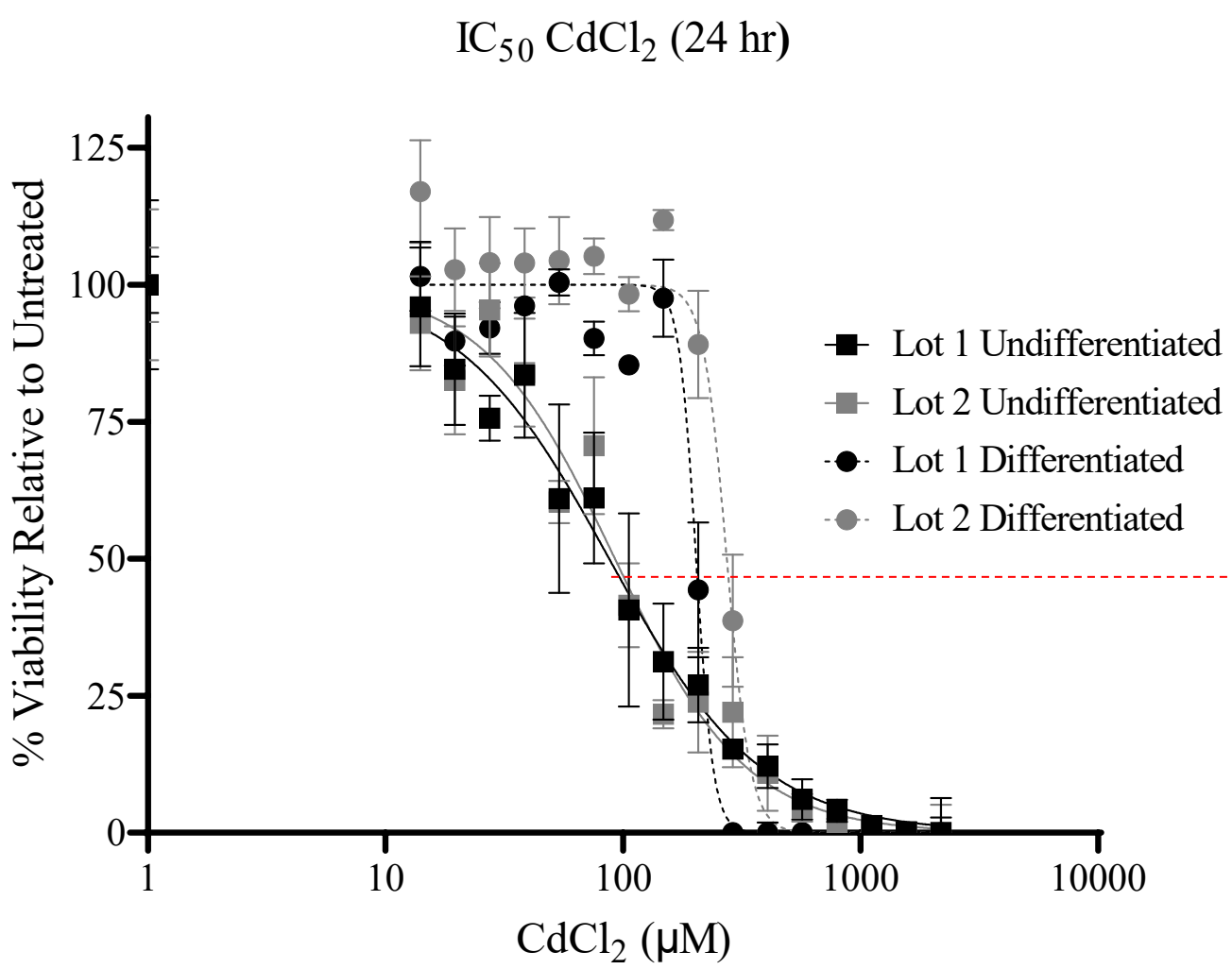
4-5 Weeks

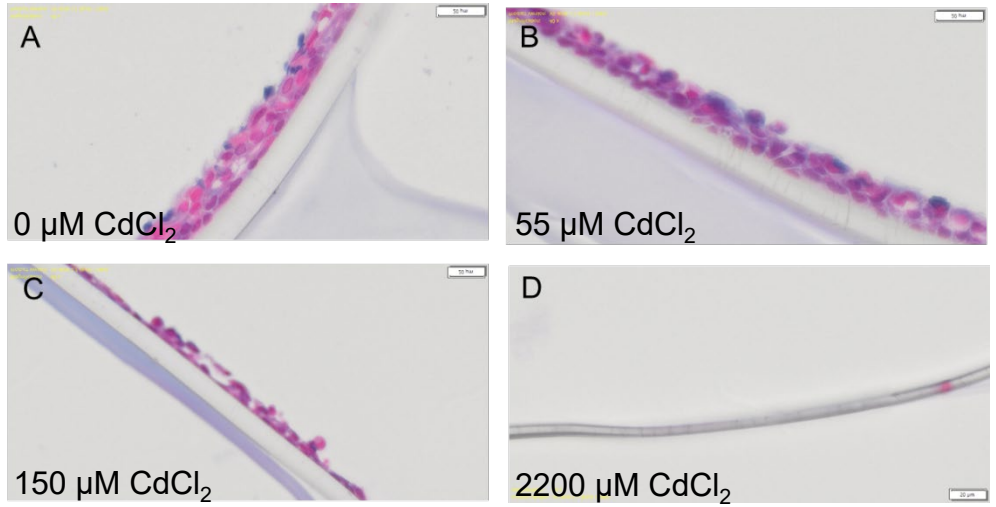


Begin studies following
epithelial differentiation



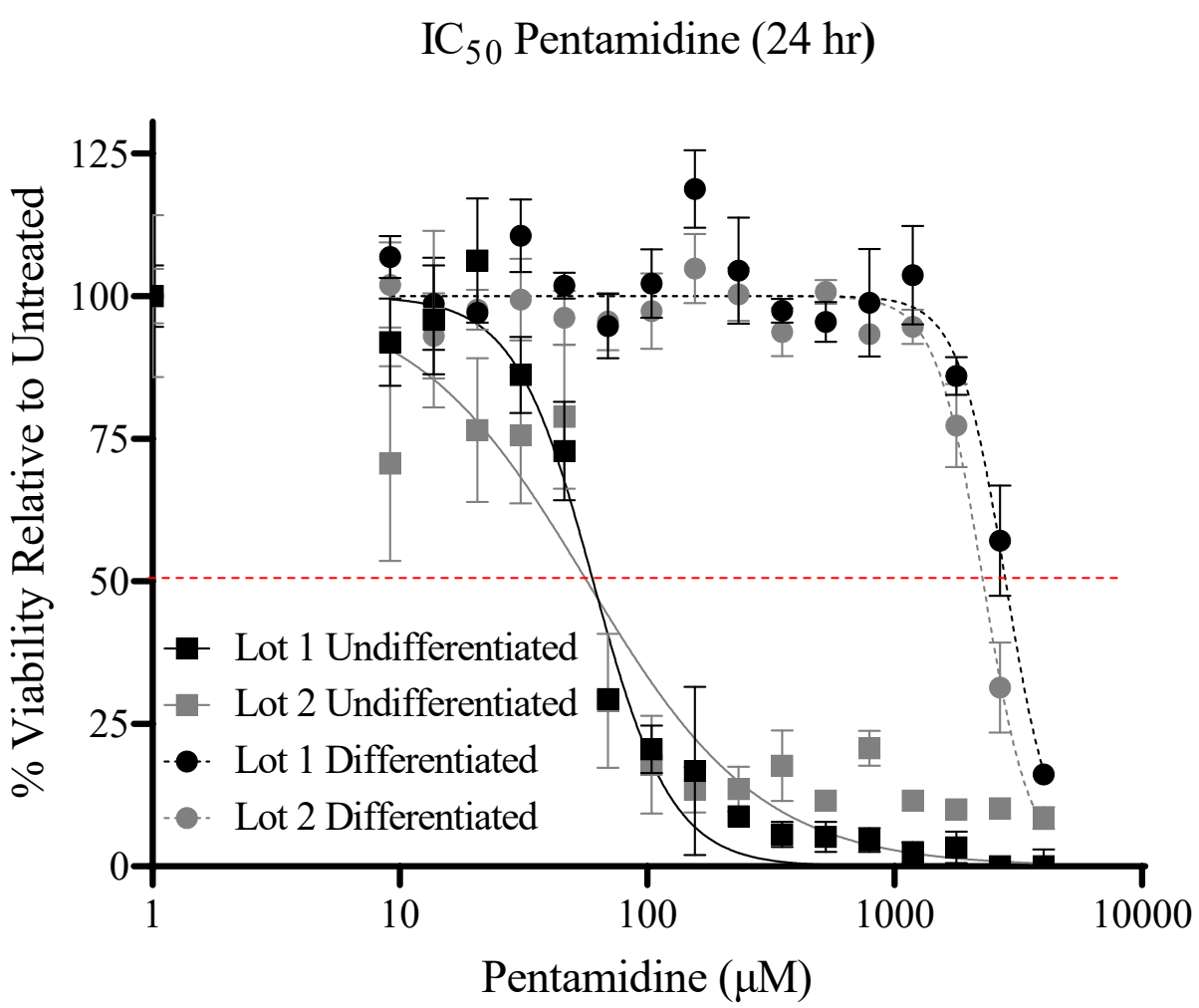
24 Hr $CdCl_2$: IC_{50} curves

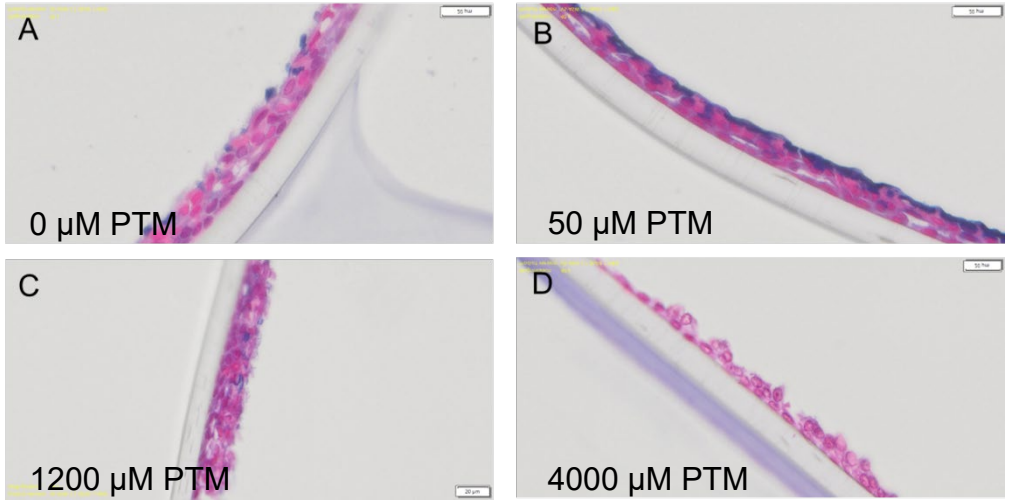




Cadmium Chloride IC_{50} values (µM)	
Sample Name	**IC_{50} Value (µM)**
Undifferentiated Lot 1	87.47 ± 10.8
Undifferentiated Lot 2	92.49 ± 10.8
Differentiated Lot 1	203.1 ± 7.2
Differentiated Lot 2	273.7 ± 12.3

24 Hr pentamidine (PTM): IC_{50} curves

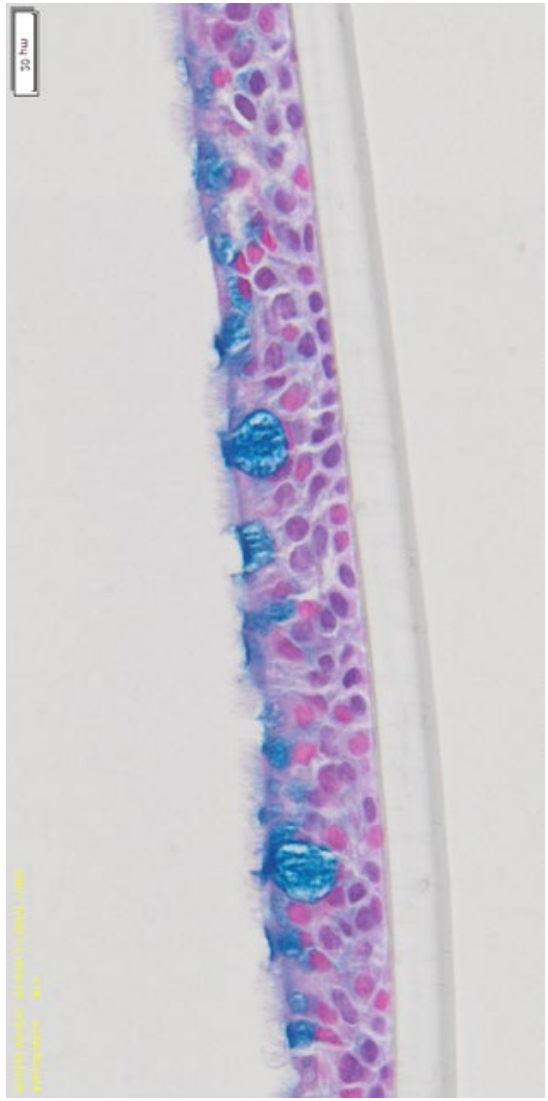


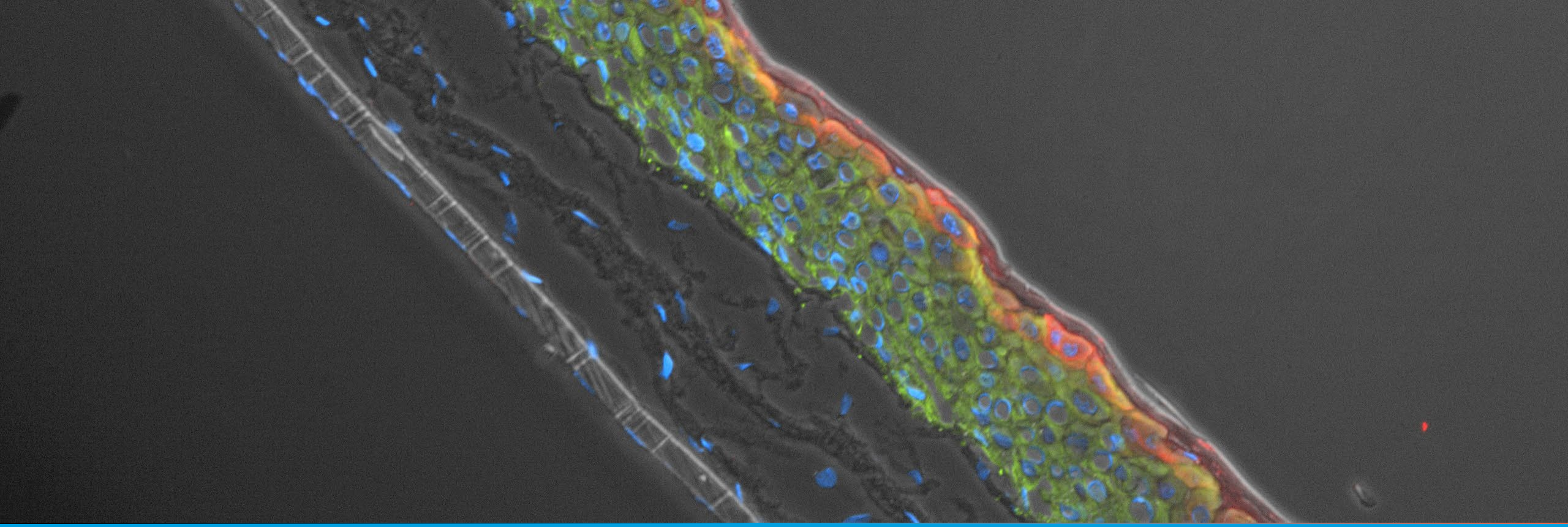


Pentamidine IC_{50} values (µM)	
Sample Name	**IC_{50} Value (µM)**
Undifferentiated Lot 1	60.4 ± 5.5
Undifferentiated Lot 2	57.1 ± 13.5
Differentiated Lot 1	2811 ± 200.5
Differentiated Lot 2	2279 ± 113

Summary of airway models

- ALI models built using primary human bronchial epithelial cells and hTERT-immortalized fibroblasts showed:
  - Cytotoxic and inflammatory response to multiple toxicants
  - Dose-dependent response
  - Consistency between different lots
- Fully differentiated lung airway models showed higher tolerance to selected compounds, compared to conventional 2D culture
  - $CdCl_2$
  - Pentamidine
- Lung airway model using primary human airway epithelial cells can be a reliable model for pulmonary toxicity assays
  - Viability
  - Cell cytoxicity assay
  - (TEER assay) Barrier function
  - IL-8

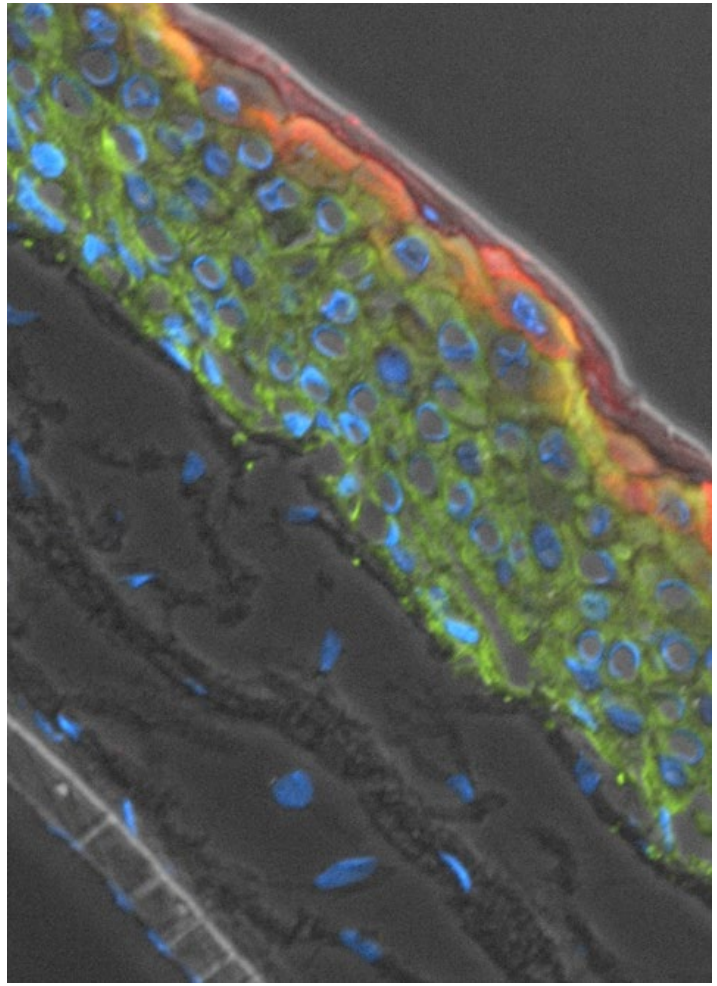




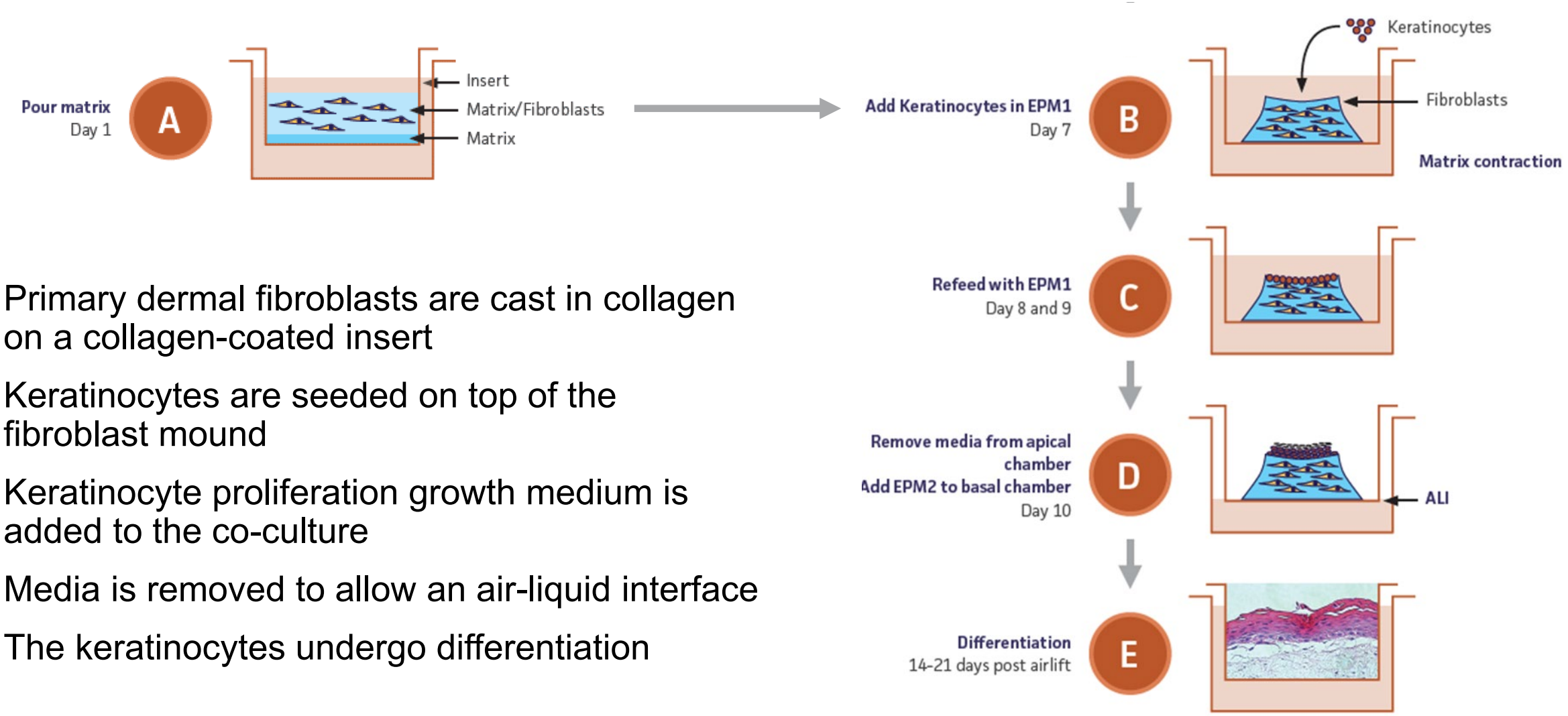
Dermal Models and Functionality

ATCC Epidermal Models

- ATCC provides several dermal cell types to support R&D efforts
- From basic research through discovery and development to product testing
  - Primary cells
    - Primary Epidermal Keratinocytes, Adult or Neonatal
    - Primary Epidermal Melanocytes, Adult Neonatal
  - hTERT-immortalized primary cells
    - Keratinocytes, Ker-CT, Adult
    - Melanocytes, Adult Female Caucasian or Neonatal Male Asian Donor
  - Also provide primary and hTERT fibroblasts and microvascular endothelial cells
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalable to research needs
  - Biological relevancy



Overview of Co-culture of Keratinocytes and Fibroblasts



A. Primary dermal fibroblasts are cast in collagen on a collagen-coated insert

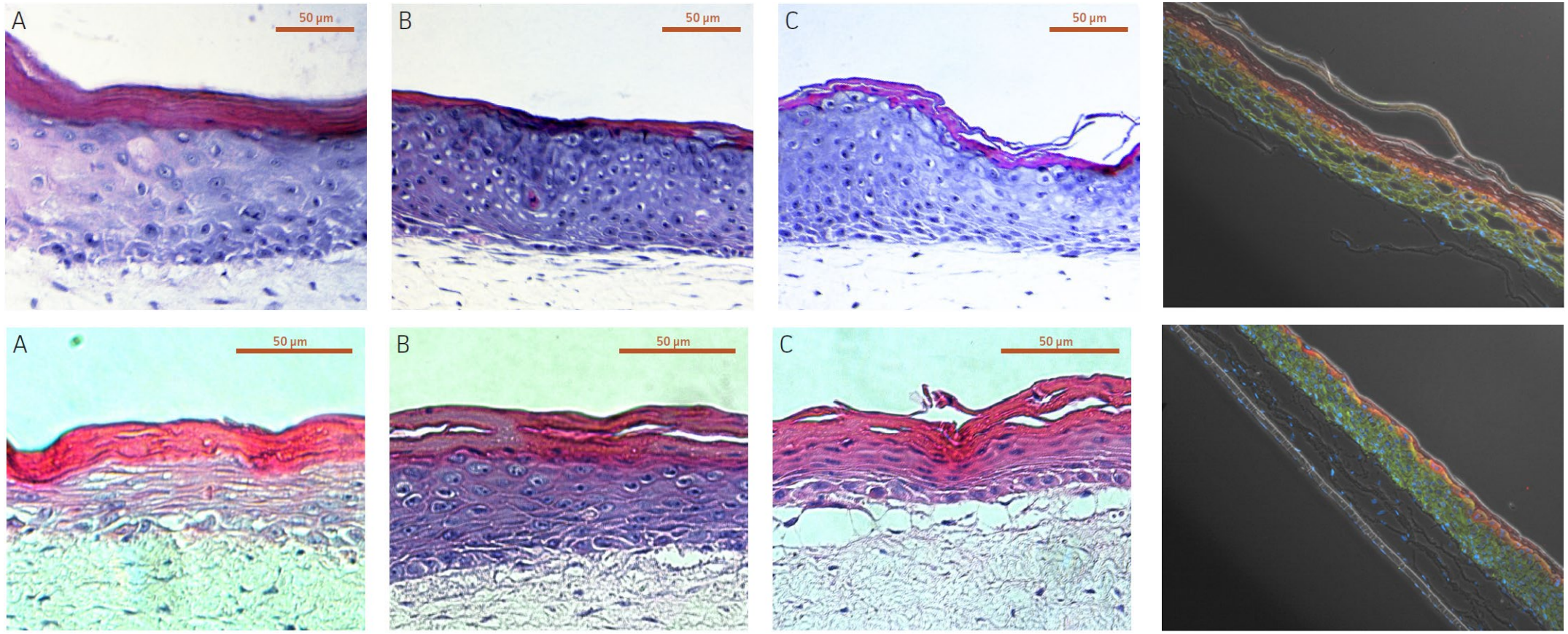
B. Keratinocytes are seeded on top of the fibroblast mound

C. Keratinocyte proliferation growth medium is added to the co-culture

D. Media is removed to allow an air-liquid interface

E. The keratinocytes undergo differentiation

Micrograph of multi-cellular dermal ALI culture featuring primary foreskin keratinocytes and Ker-CT at low and high passage



- A) primary foreskin keratinocytes at passage 2
- B) Ker-CT at passage 6
- C) Ker-CT at passage 15
- Top panels 11 days post ALI culture
- Bottom panels 21 days post ALI culture

Immunofluorescence

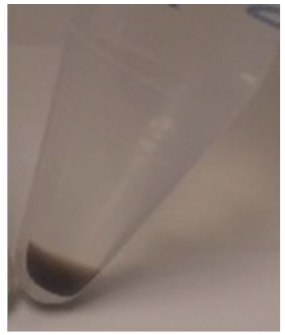
Top: Primary keratinocytes; Bottom: Ker-CT

- Green = Krt14
- Red = Filiggrin
- Blue = DAPI

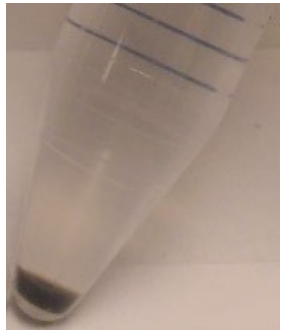
Authentication of primary and hTERT-immortalized melanocytes

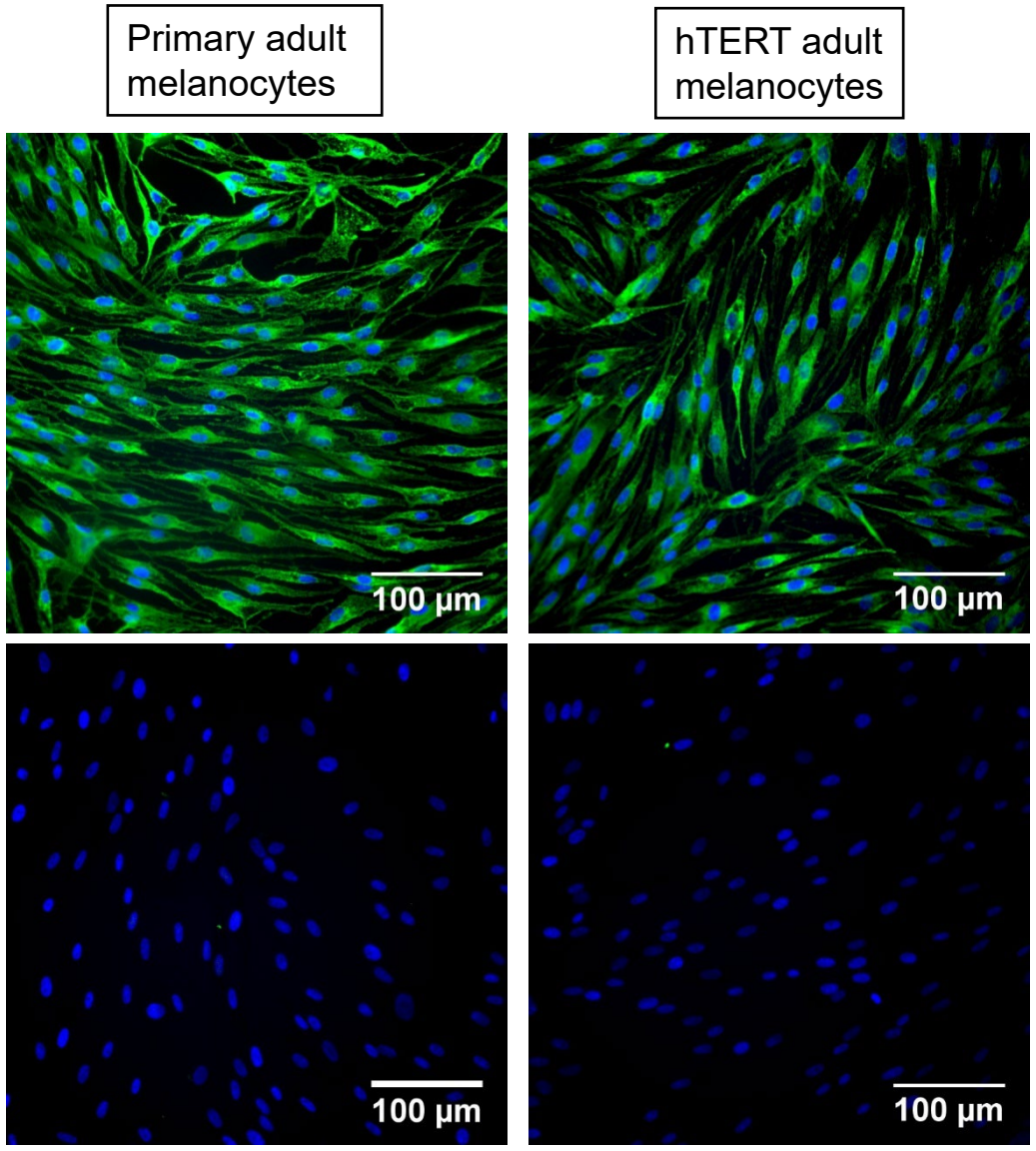
negative control
(adipose) cell pellet

Primary adult
melanocytes



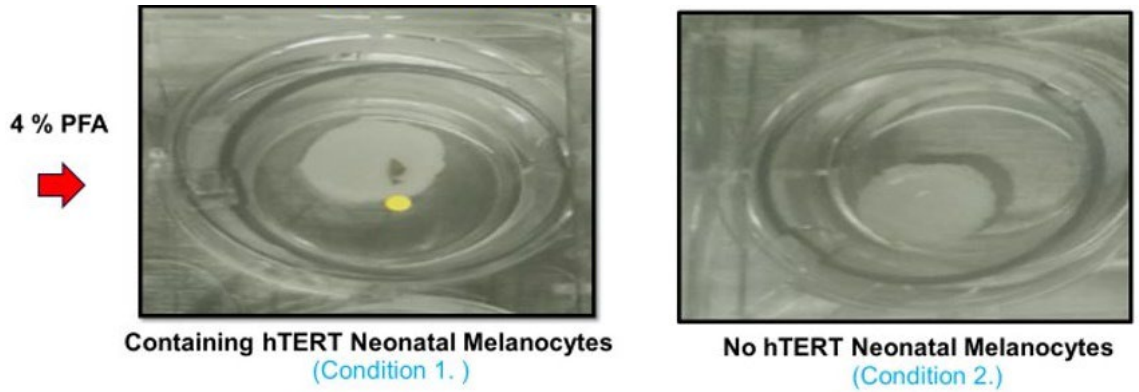
hTERT
melanocytes





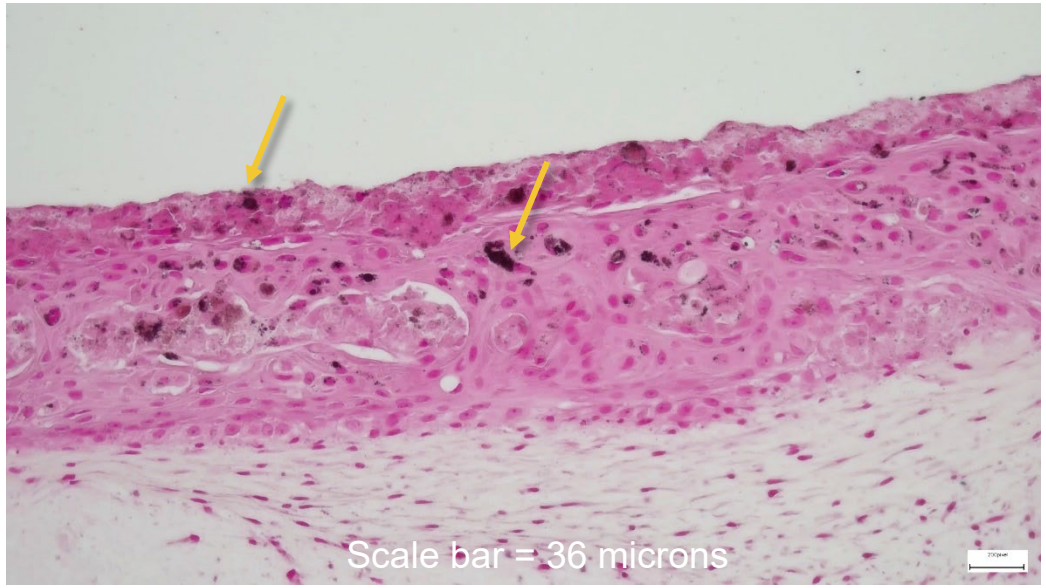
Neonatal melanocyte 3-D organotypic culture

Melanin deposits visible in 3D organotypic co-culture

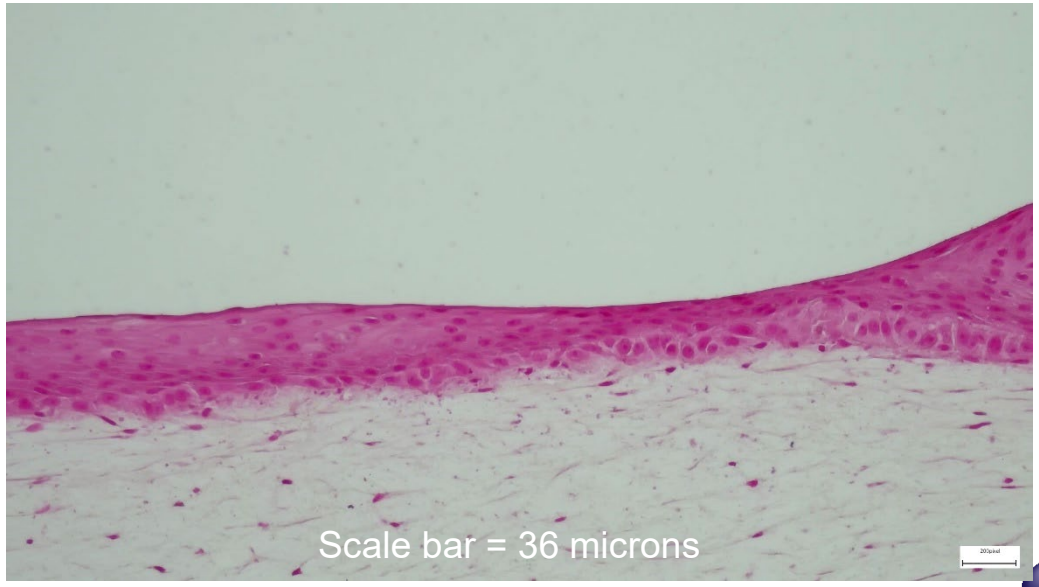


- Melanin visible in macroscopic & microscopic images of 3d cultures.
- Generally, less tissue development is observed in cultures without melanocytes.

Fibroblast/Keratinocytes + hTERT Neo Melanocytes

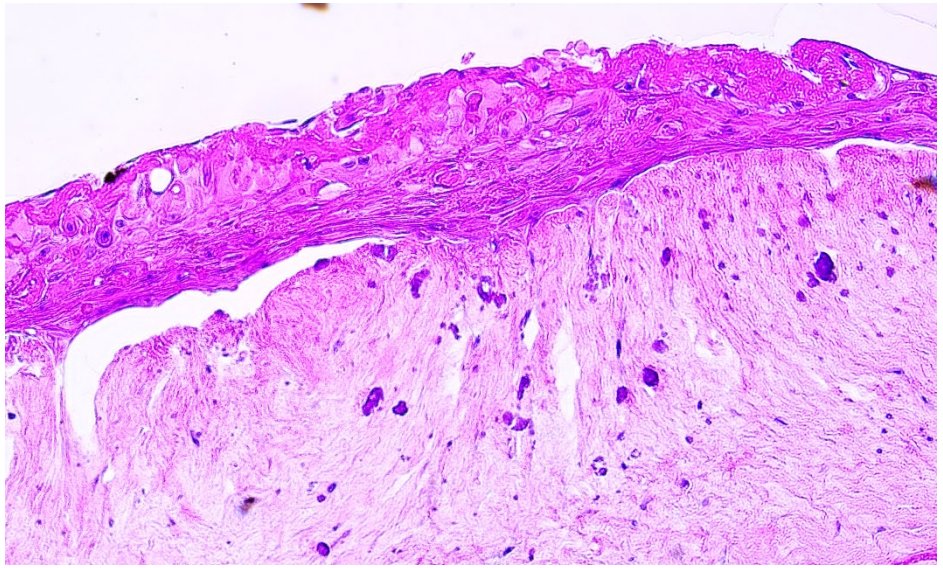


Fibroblast/Keratinocytes

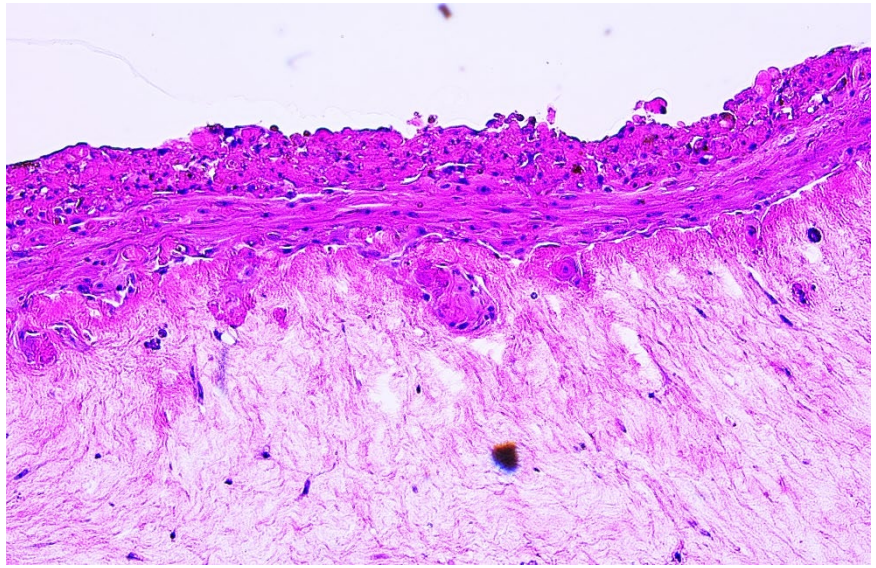


Fontana Masson Stain, 20x Brightfield, Brightness +20%

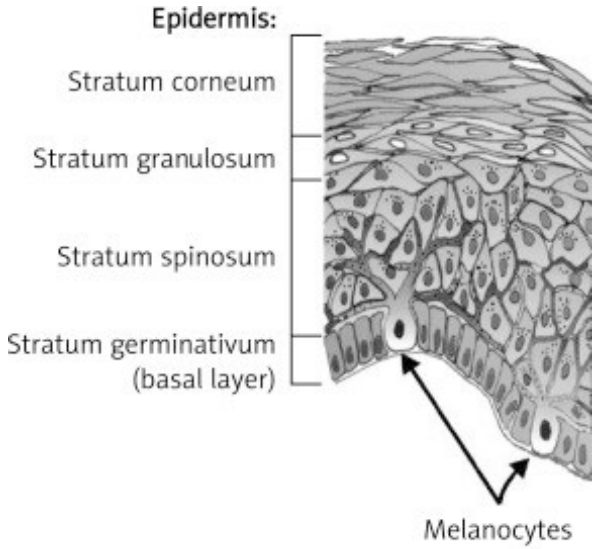
3D Dermal culture using primary human dermal cells



Primary Fibroblast/Keratinocytes



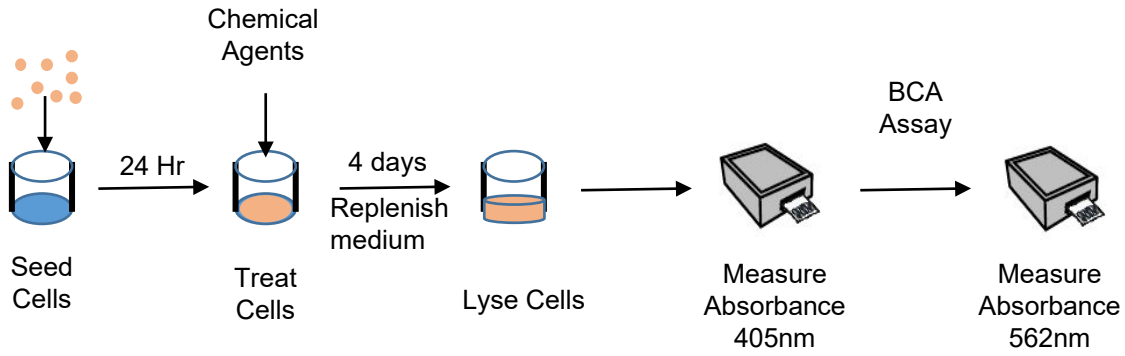
Primary Fibroblast/Keratinocytes
+ Primary Melanocytes

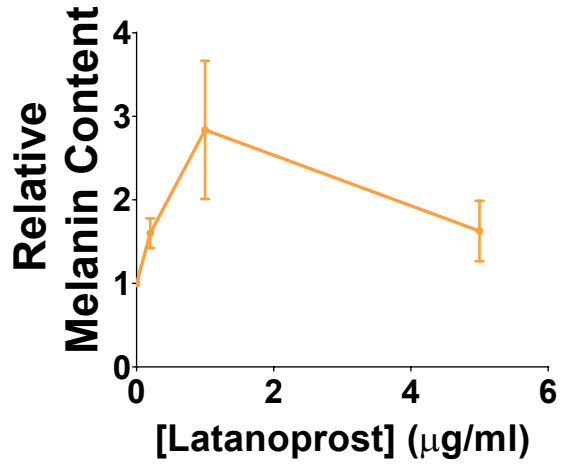


Postepy Dermatol Alergol. 2013 Feb; 30(1): 30–41.

Adult Melanocyte Stimulation and Inhibition Study

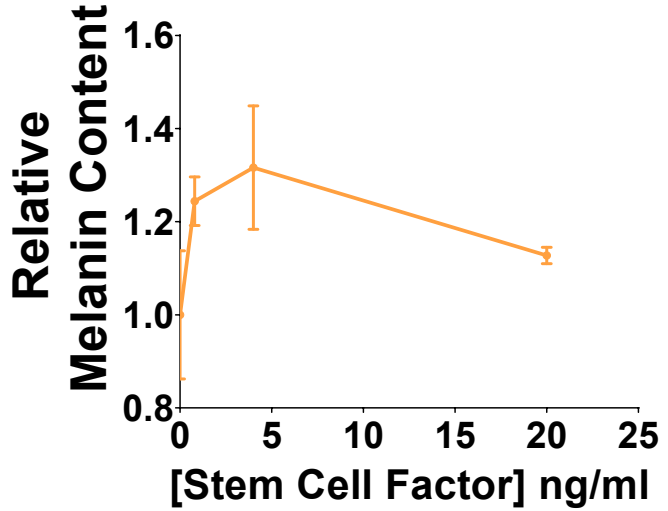
Testing responsiveness to stimulators and inhibitors of melanogenesis

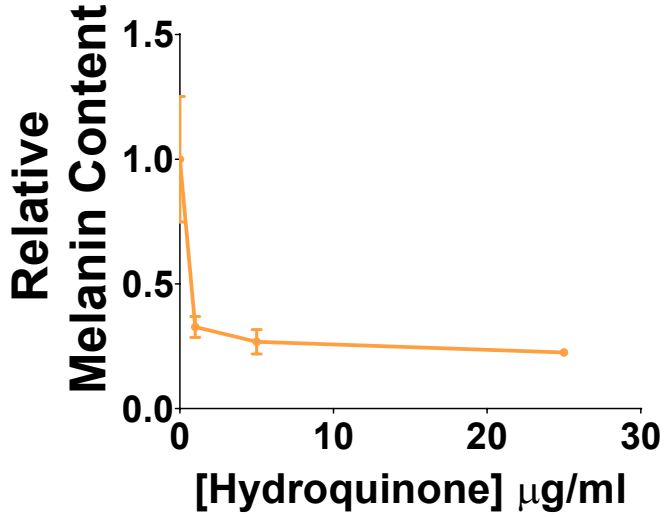




- Total protein determined by BCA assay and fitting to standard curve of 8 concentrations
- Melanin content adjusted relative to total protein and untreated control

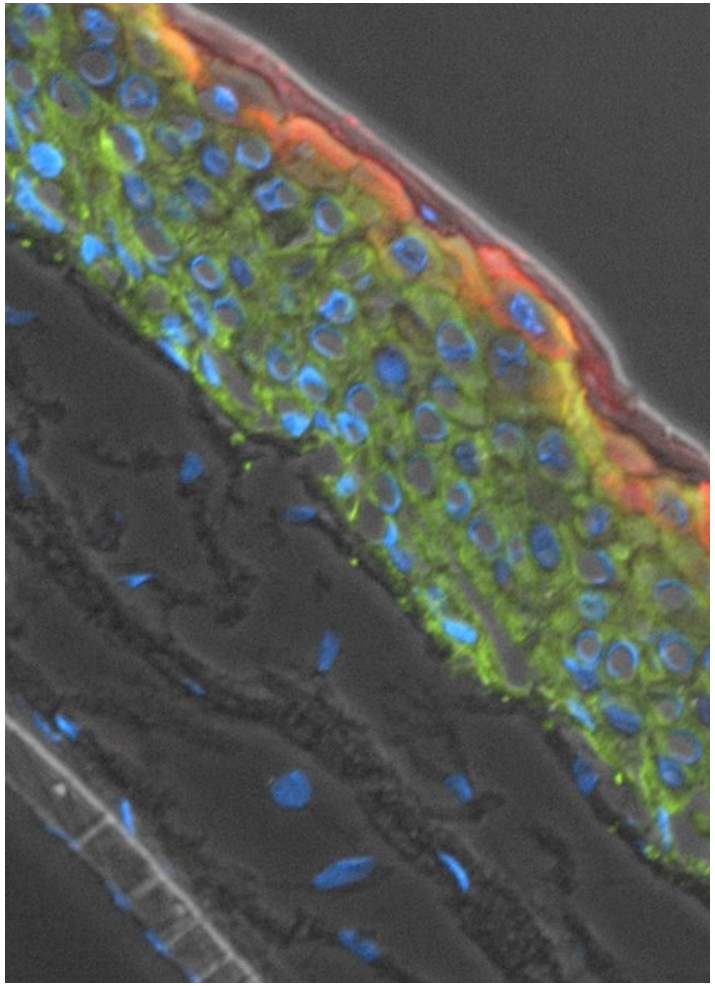
$$Rel.Mel.cont_i = \frac{\frac{A405_i}{Total\ Protein_i}}{\frac{A405_{untreated}}{Total\ Protein_{untreated}}}$$





Summary of epidermal models

- ALI models built using primary or hTERT human melanocytes, keratinocytes, and fibroblasts showed:
  - 3-D epidermal architecture
  - Appropriate marker expression
- Primary or hTERT human melanocytes incubated introduced into ALI epidermal culture
  - Aided epidermal development
  - Deposited melanin
- Melanin production in primary or hTERT-immortalized primary cells was:
  - Increased by secretagogues such as latanoprost and stem cell factor
  - Inhibited by hydroquinone and kojic acid

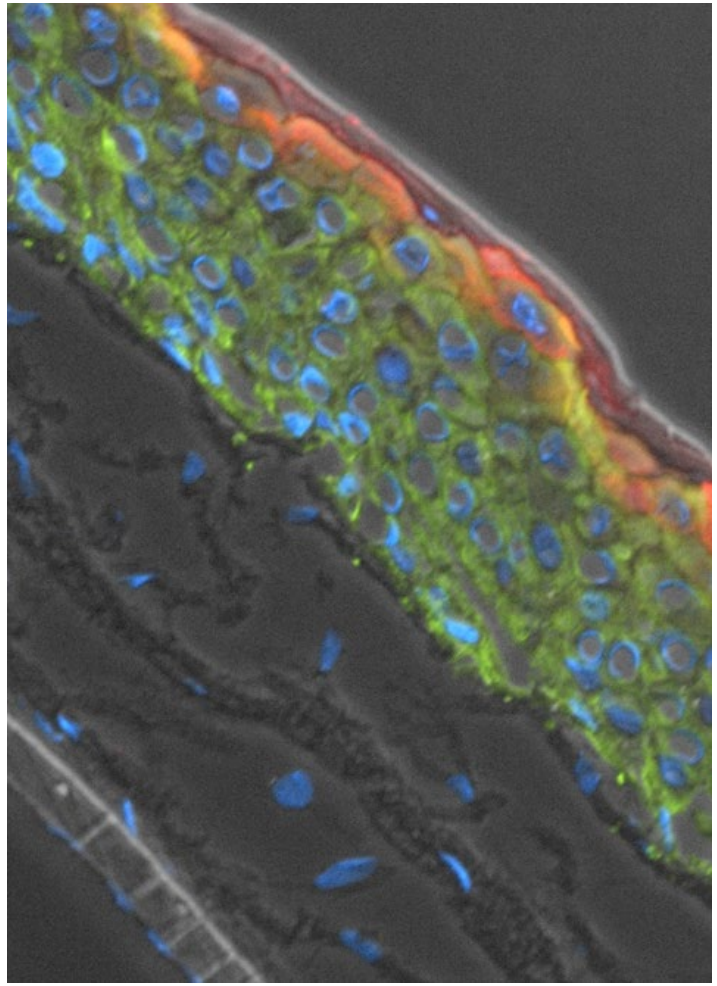


ATCC

Summary and resources

- ATCC offers comprehensive solutions for in vitro toxicology
- From basic research through biomaterial candidate discovery and development to product testing, ATCC offers a variety of cell models for toxicology research:
  - Continuous cell lines
  - Human primary cells
  - hTERT-immortalized primary cells
- hTERT immortalized primary cells provide primary cell functionality with continuous cell line longevity
- hTERT cells alone or in combination with other cells are a user-friendly solution for building reliable cell models for toxicity studies
- Multiple primary cell and hTERT-immortalized primary cell resources are available at:

www.atcc.org/tox



New products:

hTERT-immortalized Brown and White Pre-adipocytes

- Can be differentiated into adipocytes
- Useful for studying metabolic diseases, inflammation, and cancer

BMI1-immortalized Pulmonary Artery Endothelial Cells

- Can form capillary-like tubules on Cell Basement Membrane
- Useful for studying cardiovascular toxicity

Checkpoint Luciferase Reporter Cells

- Enables screening of checkpoint inhibitor molecules
- Wide range of targets such as PD-L1/2, CD-155, B7-H3, and PD-1
- Luciferase will be expressed under the control of GAS or NFAT

Human Cancer Models Initiative (HCMI)

- 2-D and 3-D patient-derived models available
- Novel models such as organoids and conditionally reprogrammed cells
- Diverse genetic backgrounds of the same cancer types
- Culturing protocols and organoid growth kits