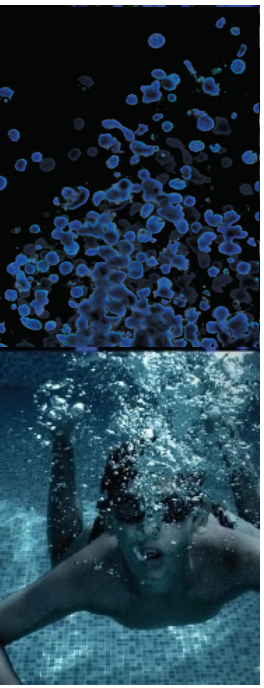
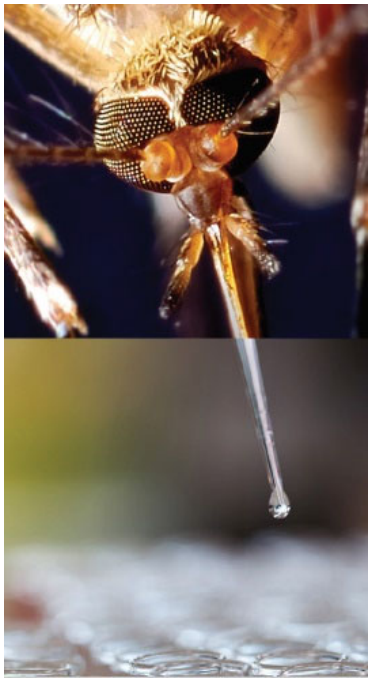
Elevate Your Toxicity Assays: New Models with Biological Relevance and Predictability

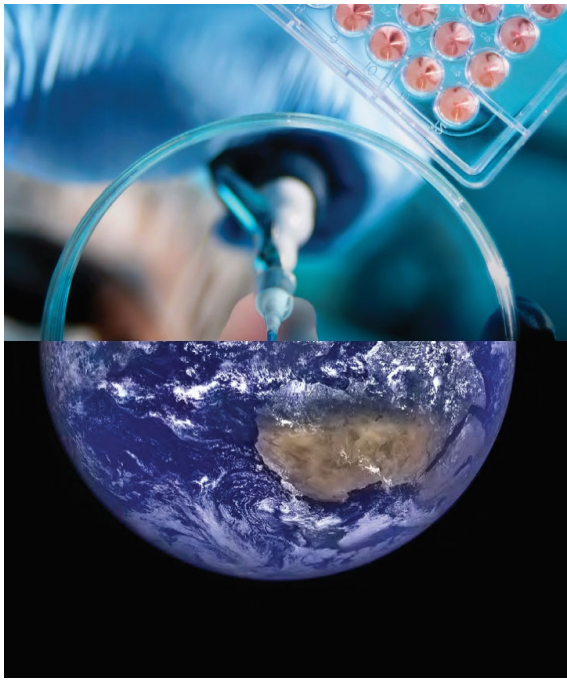


Kevin Grady, BS
Senior Product Line Business Manager, ATCC

Kevin Tyo, PhD
Scientist, ATCC

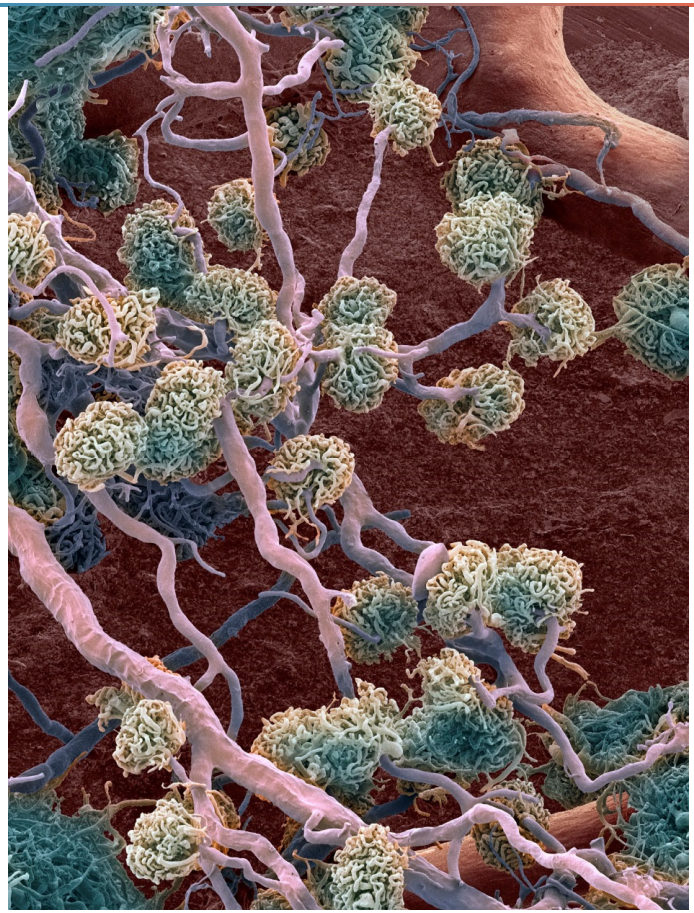
Credible Leads to Incredible™





Agenda

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



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 gene editing, differentiated stem cells, advanced 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

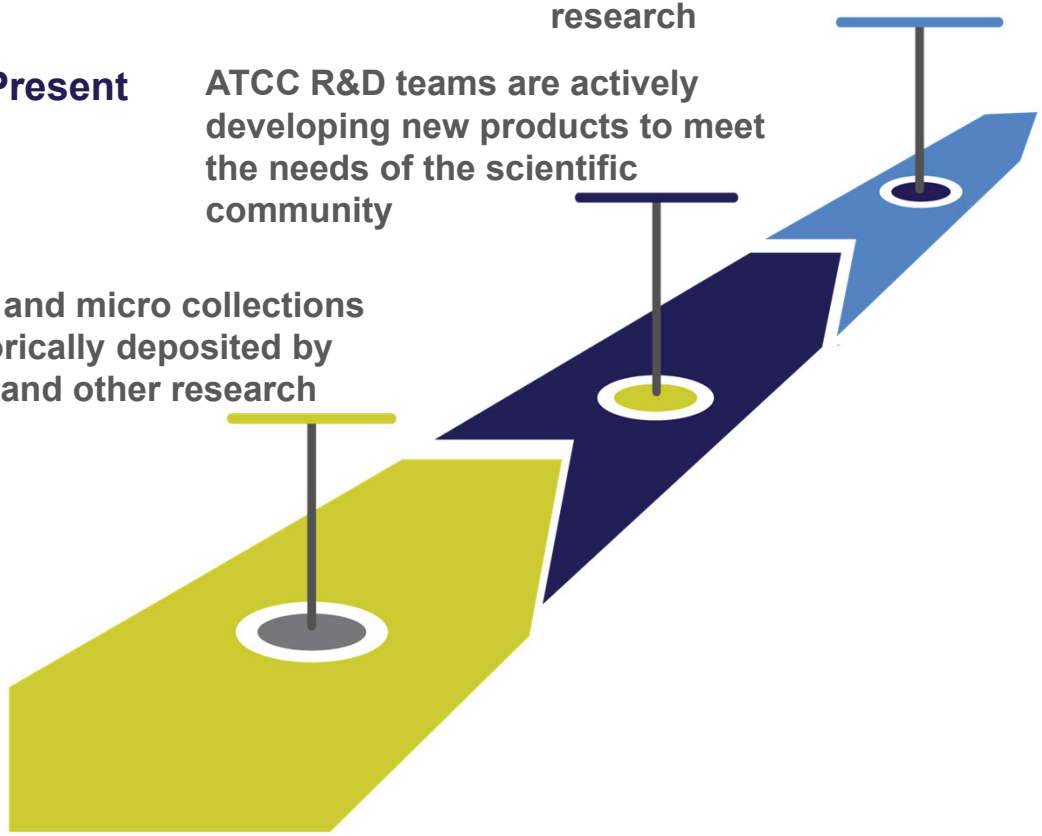
ATCC

Modernization of the ATCC portfolio

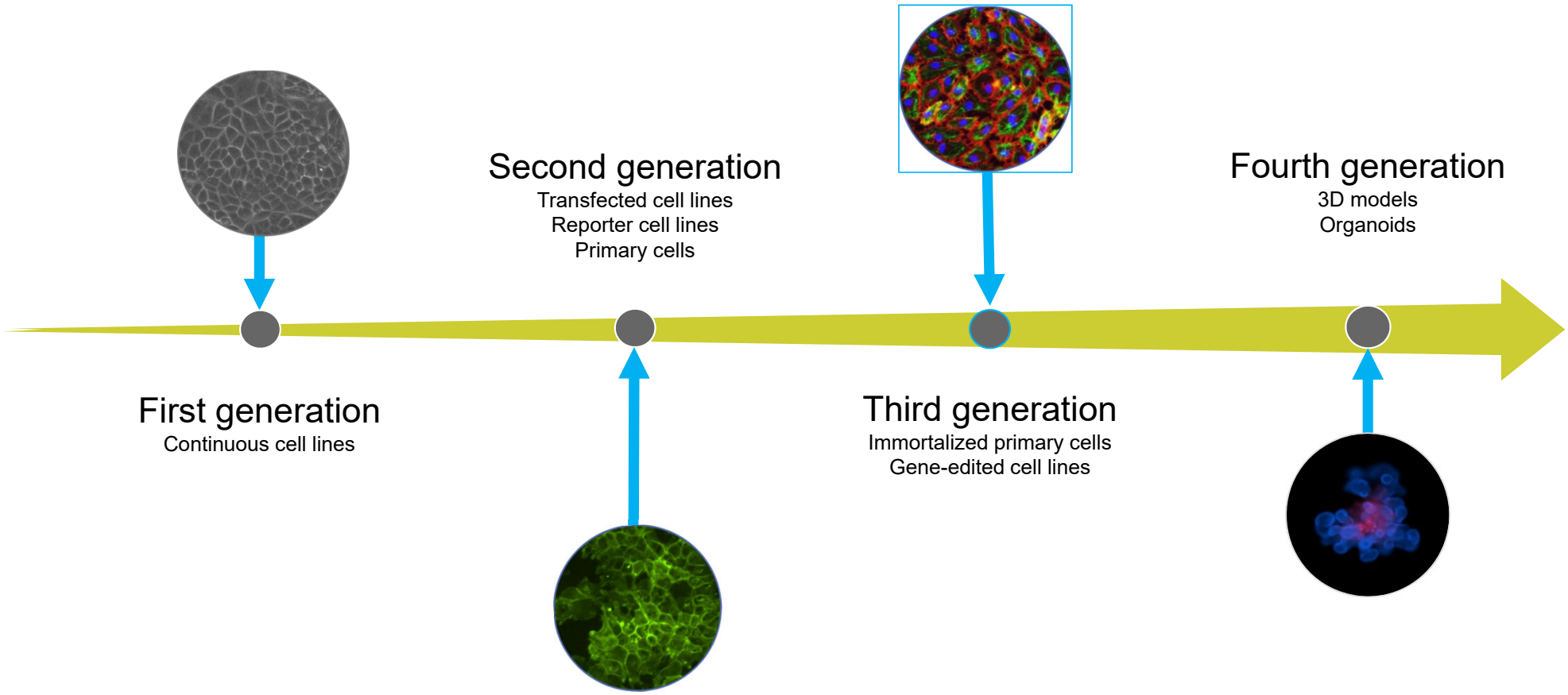
Ensuring the Future — ATCC is investing in key technologies to ensure its products and services remain the definitive standards in biological research

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

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



Evolution of in vitro cell models



Characteristics of various cell models

	Continuous (cancer) cell lines	Primary cells	hTERT-immortalized primary cells
Mimic *in vivo* characteristics	+	++++	+++
Proliferative capacity	+++	+	+++
Experimental reproducibility	+++	+	+++
Predictability in toxicological studies	+	+++	+++
Genomic stability	Aneuploid	Diploid	Diploid/near diploid
Supply	+++	+	+++
Cost	+++	+	++
Ease of use	+++	+	++

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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.

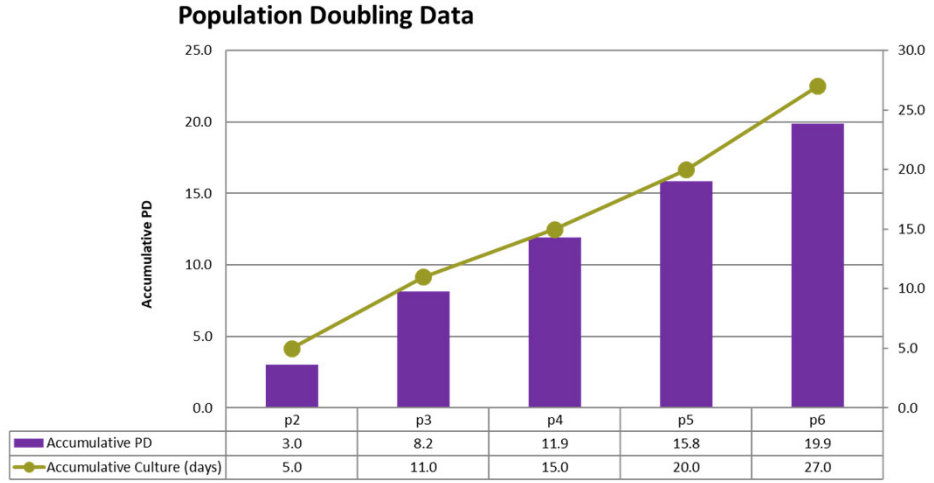
Growth

- Supplied at P0 or P2
- At least ≥ 10 (usually ≥ 15) doublings guaranteed

Characterization

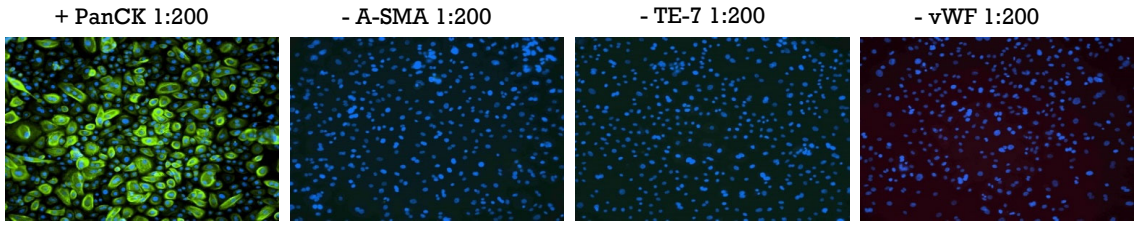
- Bio-functional testing
- Positive and negative markers confirmed via ICC or FACS

Bladder Epithelial Cells(A/T/N)

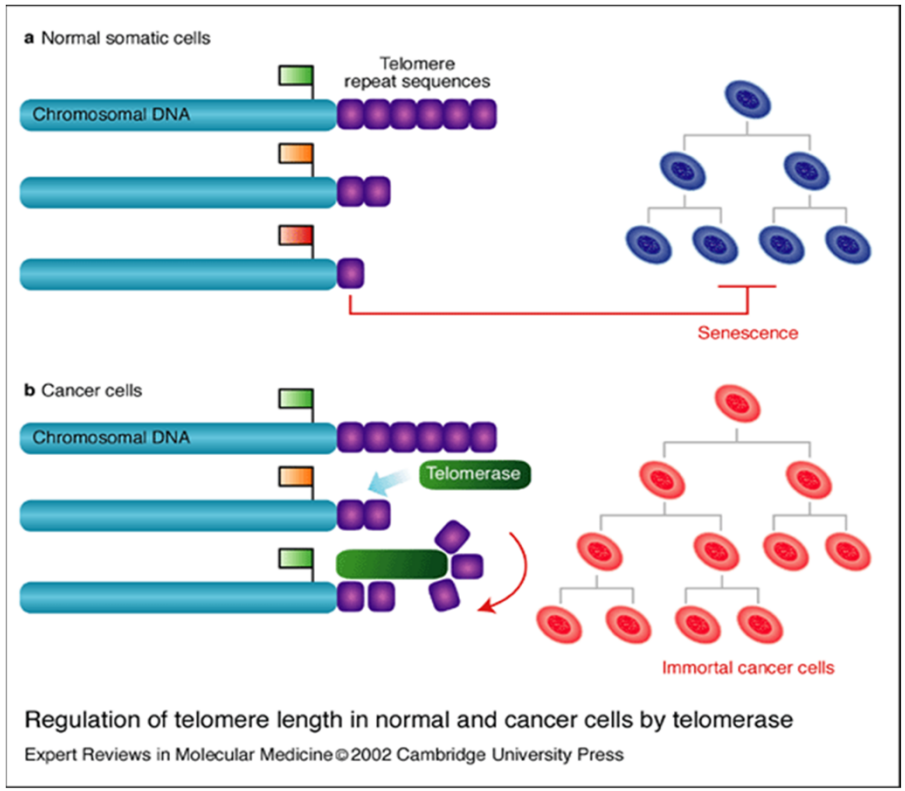


	p2	p3	p4	p5	p6
Accumulative PD	3.0	8.2	11.9	15.8	19.9
Accumulative Culture (days)	5.0	11.0	15.0	20.0	27.0

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

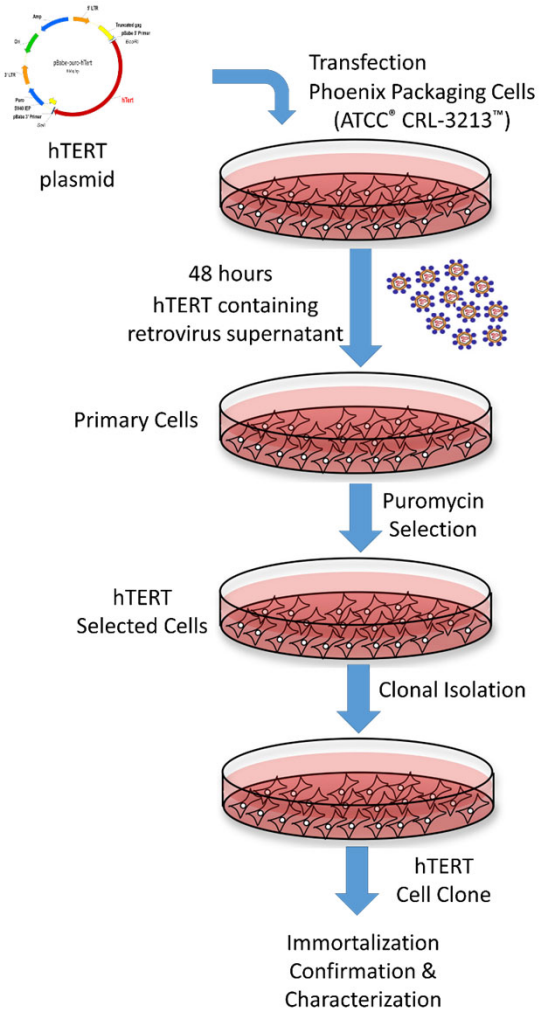


hTERT immortalization technology



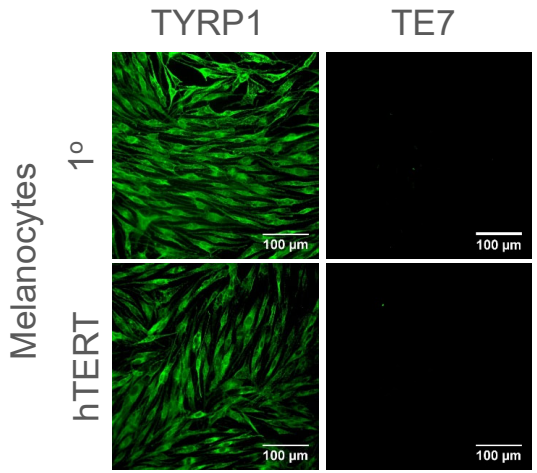
Regulation of telomere length in normal and cancer cells by telomerase

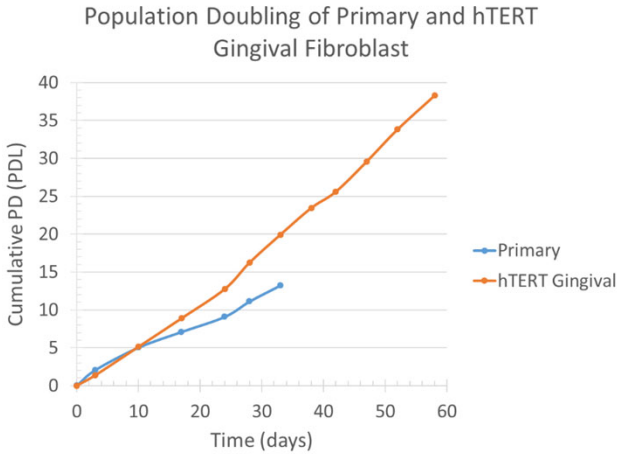
Expert Reviews in Molecular Medicine ©2002 Cambridge University Press

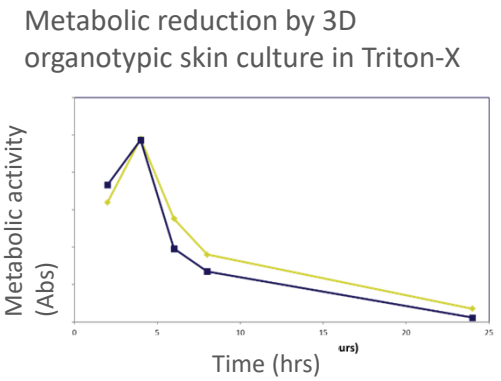


hTERT-immortalized cells – Key characteristics

- Growth
  - Cells retain replicative capacity (“immortalized”)
  - Population doubling rate is comparable to primary cells
- Morphology and marker expression
  - Similar to primary cells
- Toxicology responses
  - Analogous to primary cells



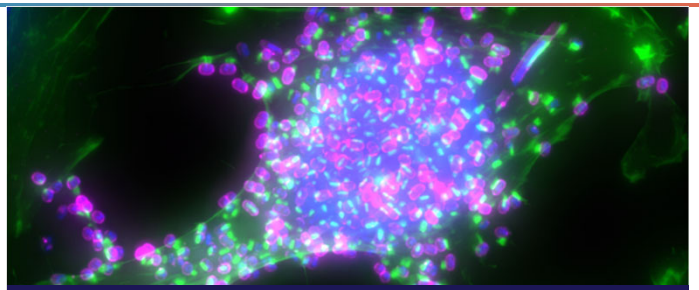




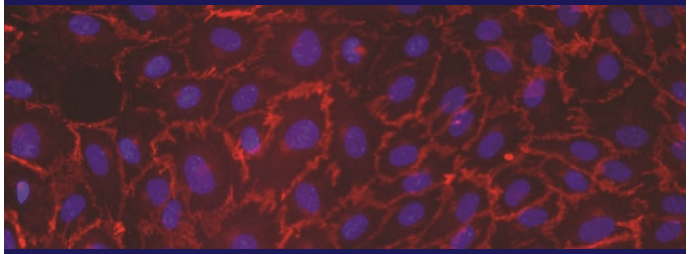
ATCC products for toxicology

- ATCC is the complete solution supplier for toxicology
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - Primary cells
  - hTERT-immortalized primary cells

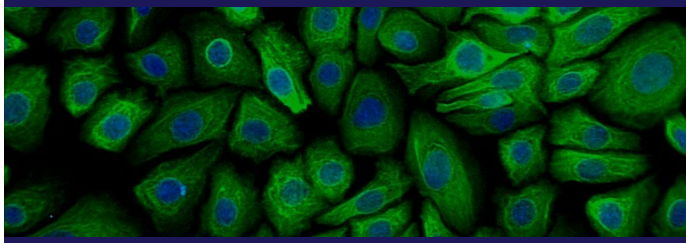
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalability into all aspects of the toxicology workflow
  - Biological relevancy



Continuous cell line: HeLa (ATCC® CCL-2™)



Primary: Umbilical Endothelial Cells (ATCC® PCS-100-010™)



Immortalized lung cells; NuLi-1 (ATCC® CRL-4011™)

Airway Models and Functionality

ATCC®

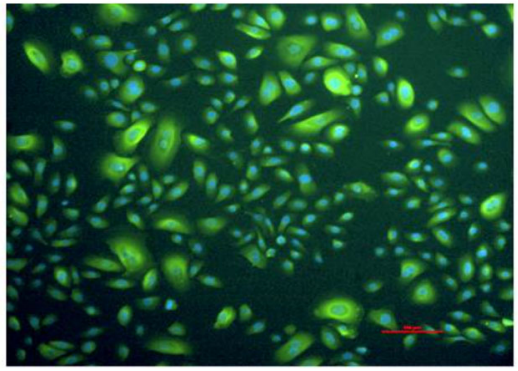
ATCC products for airway models

- Primary cells
  - Bronchial/tracheal epithelial cells
  - Small airway epithelial cells
  - Lobar epithelial cells
  - Lung smooth muscle cells
  - Bronchial/tracheal smooth muscle cells
  - Lung fibroblasts
  - Disease airway cells
    - Asthma, COPD, Cystic Fibrosis, Fibrosis

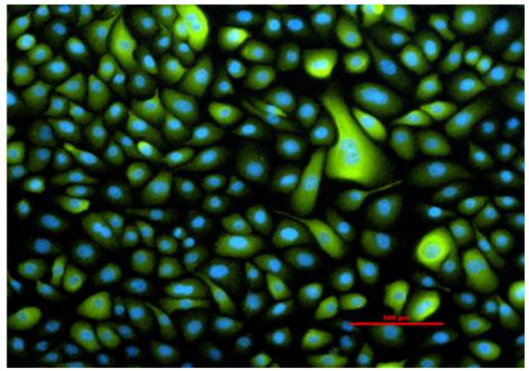
- hTERT-immortalized primary cells
  - HBEC-3KT (Bronchial epithelial cells)
  - NuLi-1 (Bronchial epithelial cells)
  - HSAEC1-KT (Small airway epithelial cells)
  - HTERT Lung Fibroblast

CCSP + DAPI

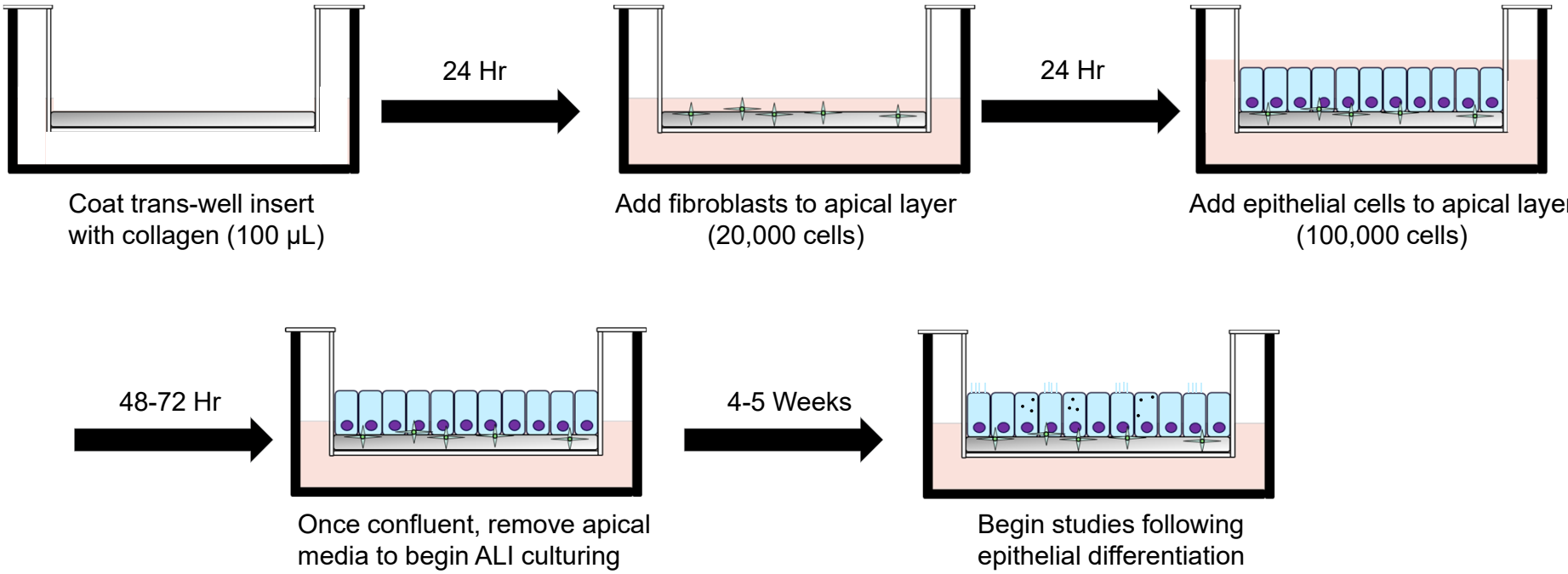
Primary Bronchial/Tracheal Epithelial Cells



HSAEC1-KT

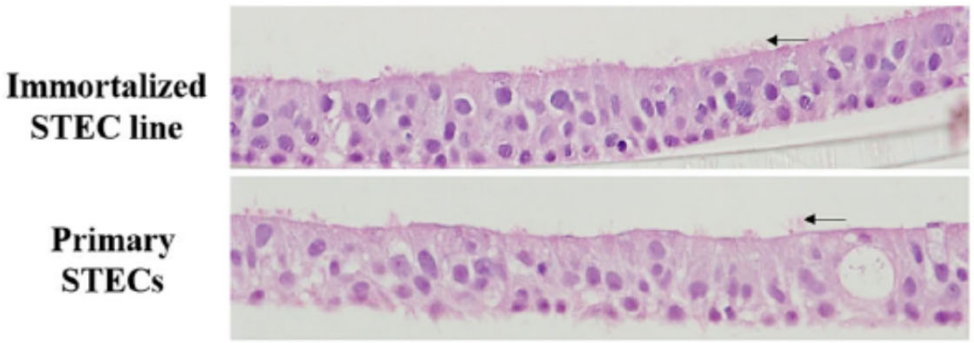


Overview of airway model fabrication

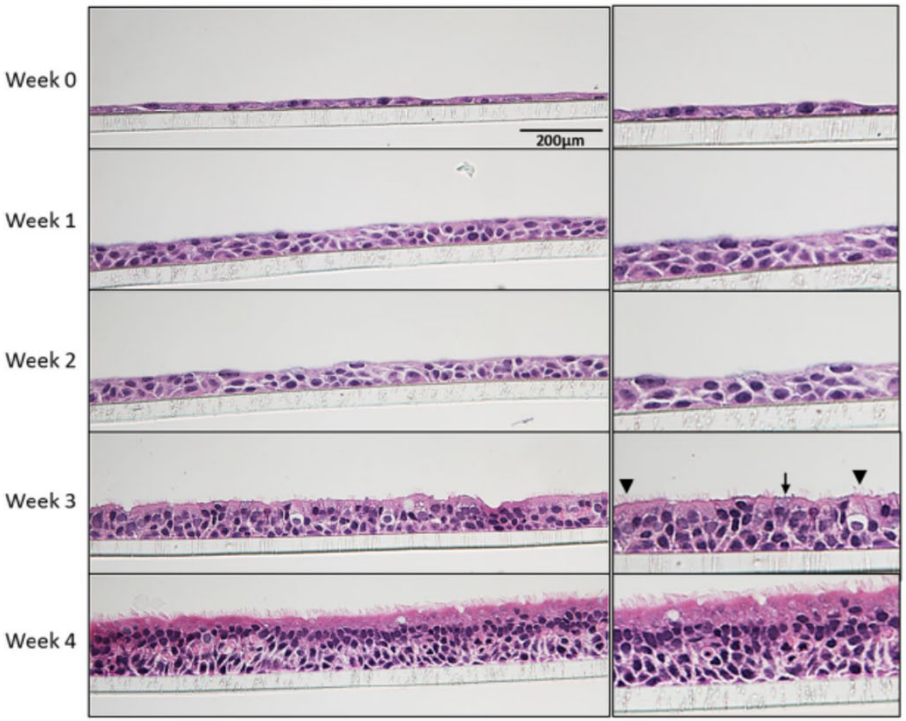


Histology images from scientific literature

Imaging is conducted using both alcian blue and hematoxylin eosin (H&E) staining on histological sections of airway models. This allows for the visual confirmation of the presence of basal, goblet, and ciliated cells.



Wang H, He L, Liu B, Feng Y, Zhou H, Zhang Z, Wu Y, Wang J, Gan Y, Yuan T, Wu M, Xie X, Feng Z. Establishment and comparison of air-liquid interface culture systems for primary and immortalized swine tracheal epithelial cells. BMC Cell Biol. 2018 Jun 28;19(1):10. doi: 10.1186/s12860-018-0162-3. PMID: 29954317; PMCID: PMC6025731.



Rayner RE, Makena P, Prasad GL, Cormet-Boyaka E. Optimization of Normal Human Bronchial Epithelial (NHBE) Cell 3D Cultures for in vitro Lung Model Studies. Sci Rep. 2019 Jan 24;9(1):500. doi: 10.1038/s41598-018-36735-z. PMID: 30679531; PMCID: PMC6346027.

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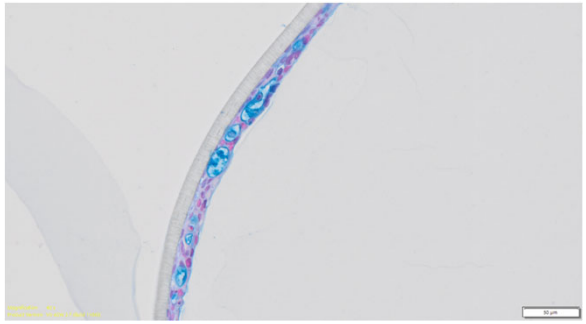
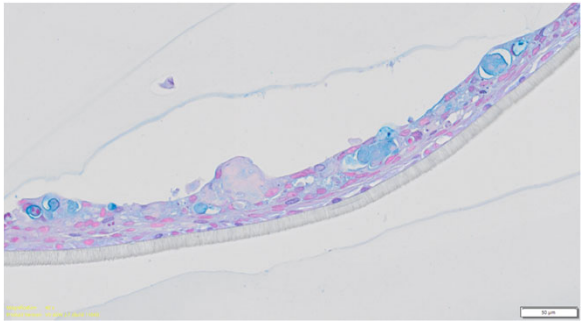
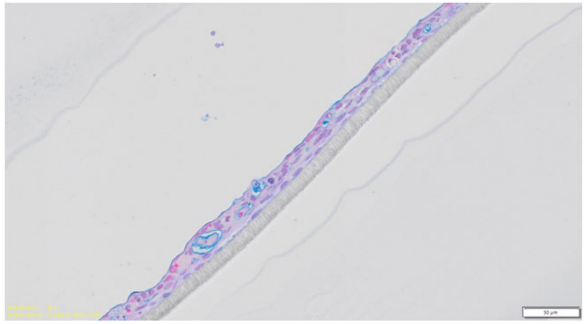
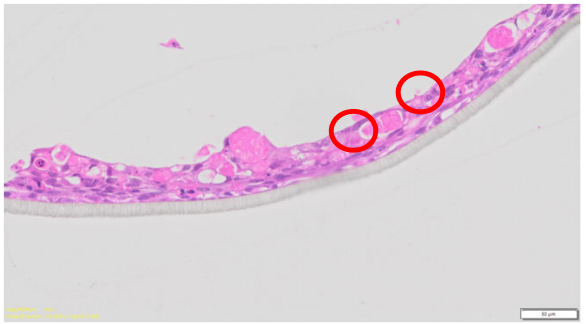
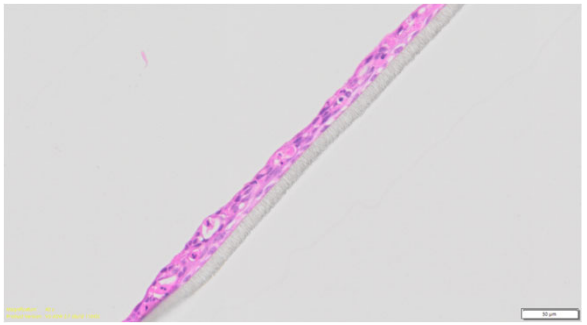
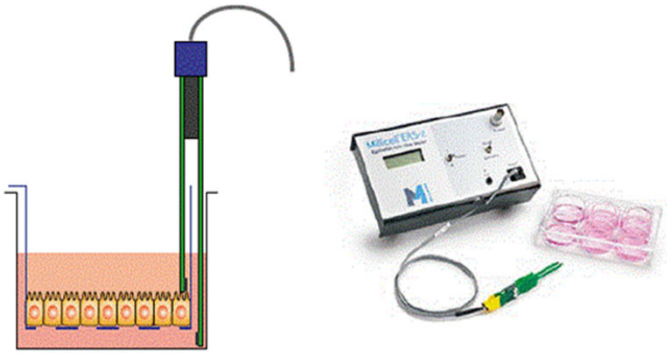
Histology images from constructed airway models

	1˚ Bronchial Epithelial Cells Only	1˚ Bronchial Epithelial + hTERT Fibroblasts	HBEC3-KT + hTERT Fibroblasts
Alcian Blue			
H&E			

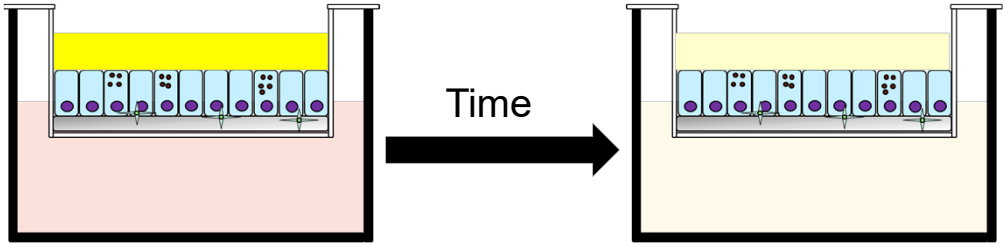
Overview of tight junction studies

Utilize EVOM ohmmeter to calculate transepithelial/transendothelial electrical resistance (TEER) of these models.



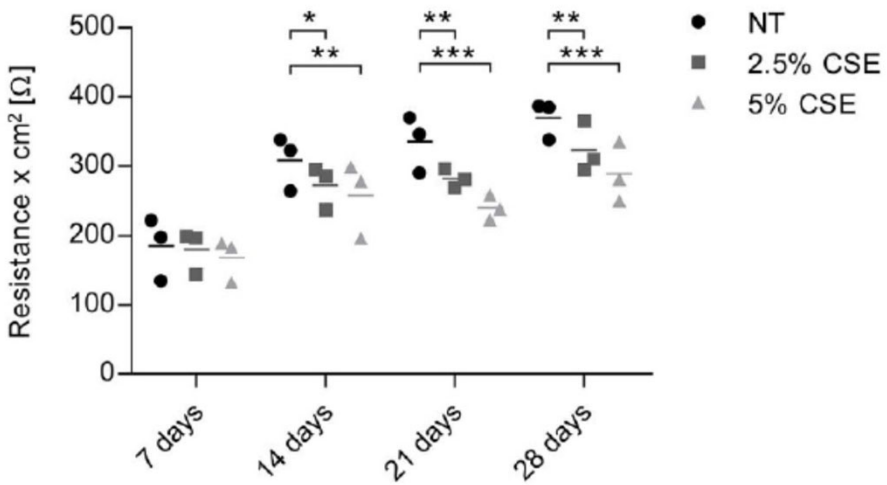
Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, Mackie A, Requena T, Swiatecka D, Wichers H, editors. The Impact of Food Bioactives on Health: in vitro and ex vivo models [Internet]. Cham (CH): Springer; 2015. PMID: 29787039.

Assess FITC dextran (40 kDa) movement from apical side of trans-well insert into the basal side. This will determine cellular tight junction formation.

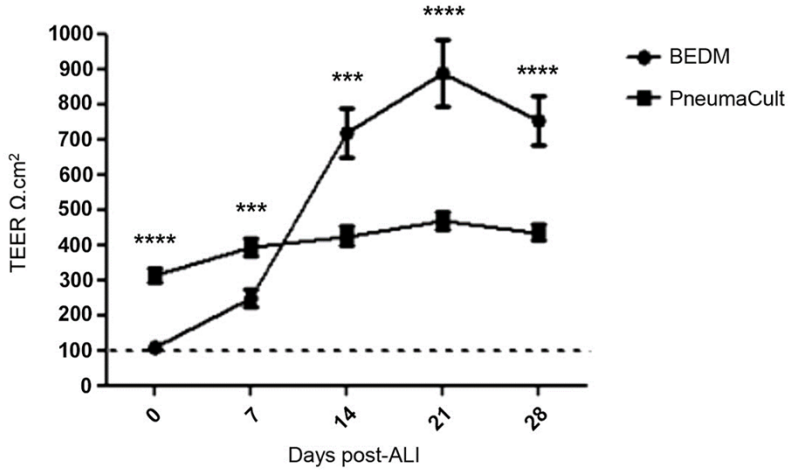


TEER values of airway models can vary

"Bronchial primary cells generally lead to TEER values of 400–4,000 ΩXcm^2."
Papazian D, Würtzen P, A, Hansen S, W, K: Polarized Airway Epithelial Models for Immunological Co-Culture Studies. Int Arch Allergy Immunol 2016;170:1-21. doi: 10.1159/000445833



Schamberger AC, Staab-Weijnitz CA, Mise-Racek N, Eickelberg O. Cigarette smoke alters primary human bronchial epithelial cell differentiation at the air-liquid interface. Sci Rep. 2015;5 8163. doi:10.1038/srep08163. PMID: 25641363; PMCID: PMC4313097.



Clarus Leung, Samuel J. Wadsworth, S. Jasemine Yang, and Delbert R. Dorscheid Structural and functional variations in human bronchial epithelial cells cultured in air-liquid interface using different growth media. American Journal of Physiology-Lung Cellular and Molecular Physiology 2020 318:5, L1063-L1073

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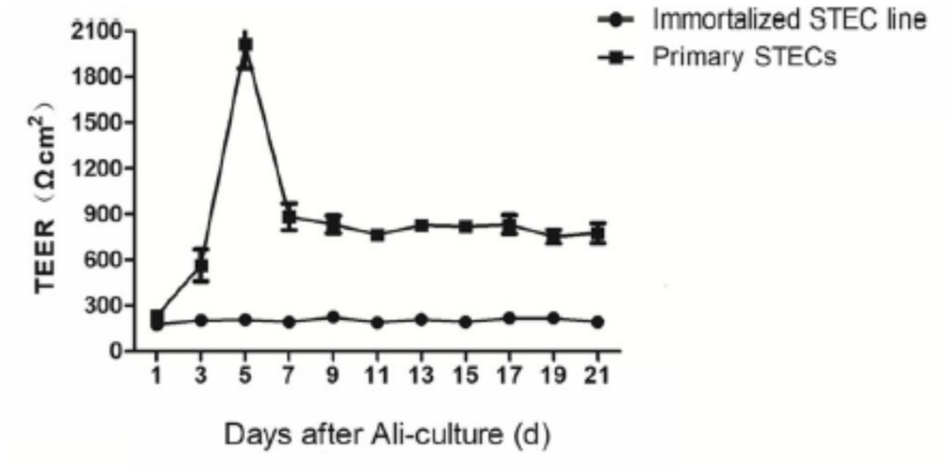
TEER values of immortalized and primary cells

"It is possible to immortalize primary human adult cells, such as with exogenous human telomerase reverse transcriptase (hTERT)…however, resulting cells can have disrupted differentiation or lack crucial biomarkers typical of an in vivo airway epithelium." Rayner, R.E., Makena, P., Prasad, G.L. et al. Optimization of Normal Human Bronchial Epithelial (NHBE) Cell 3D Cultures for in vitro Lung Model Studies. Sci Rep 9, 500 (2019). https://doi.org/10.1038/s41598-018-36735-z



Wang H, He L, Liu B, Feng Y, Zhou H, Zhang Z, Wu Y, Wang J, Gan Y, Yuan T, Wu M, Xie X, Feng Z. Establishment and comparison of air-liquid interface culture systems for primary and immortalized swine tracheal epithelial cells. BMC Cell Biol. 2018 Jun 28;19(1):10. doi: 10.1186/s12860-018-0162-3. PMID: 29954317; PMCID: PMC6025731.

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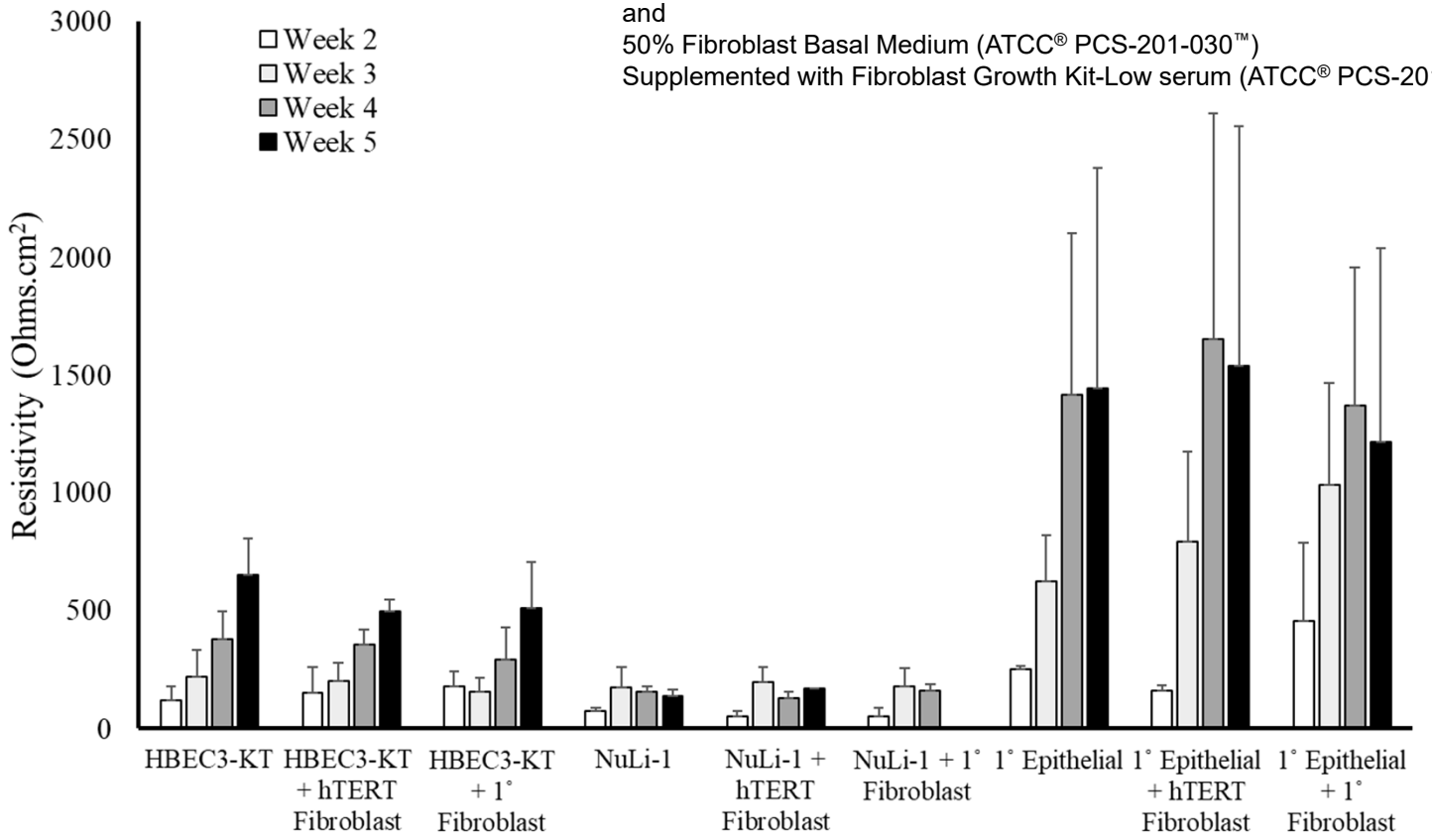
Airway model characterization: TEER measurements

Models cultured in media comprised of
50% Complete Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030™)
supplemented with Bronchial Epithelial Cell Growth Kit (ATCC® PCS-300-040™)
and
50% Fibroblast Basal Medium (ATCC® PCS-201-030™)
Supplemented with Fibroblast Growth Kit-Low serum (ATCC® PCS-201-041™)



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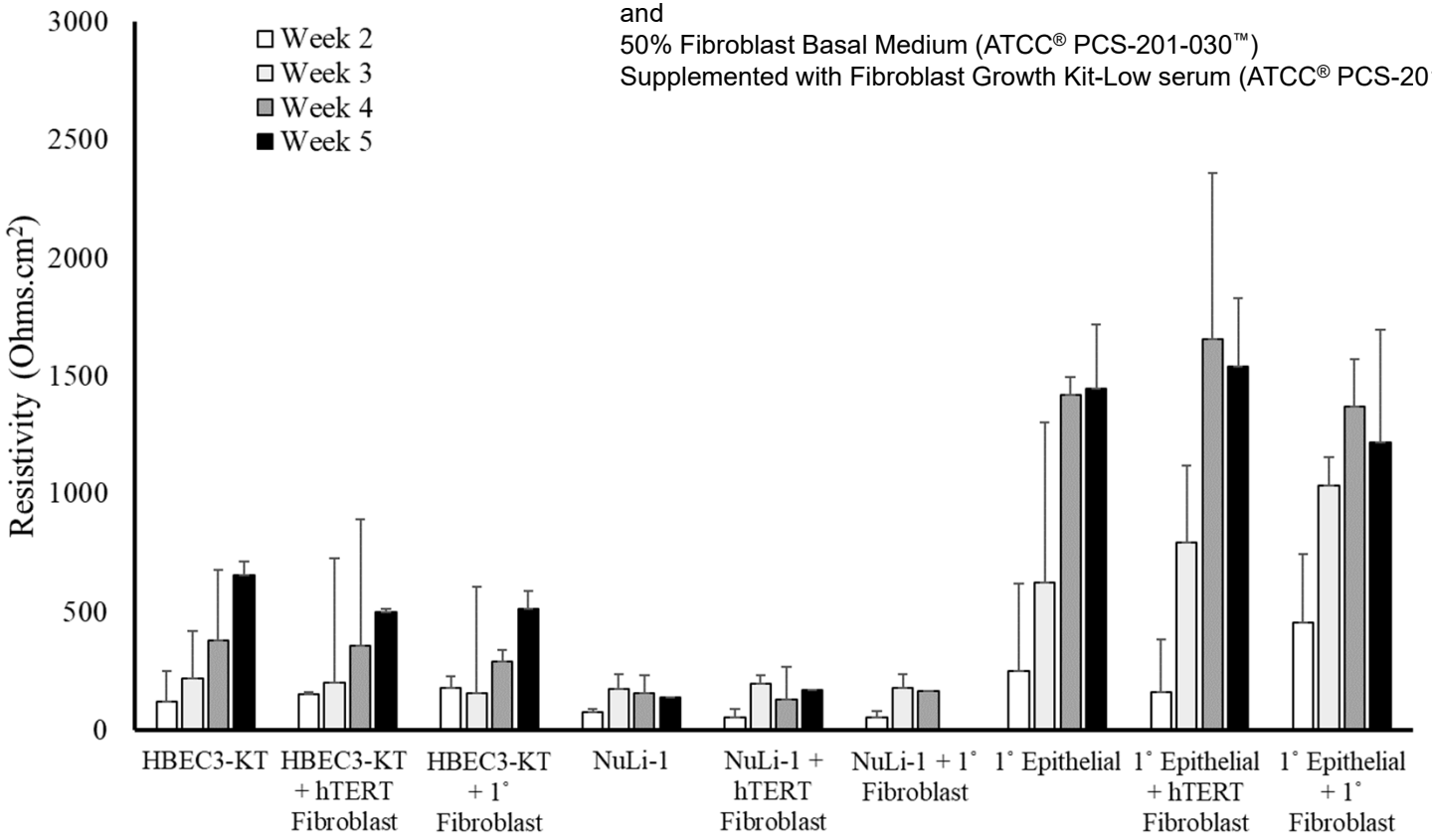
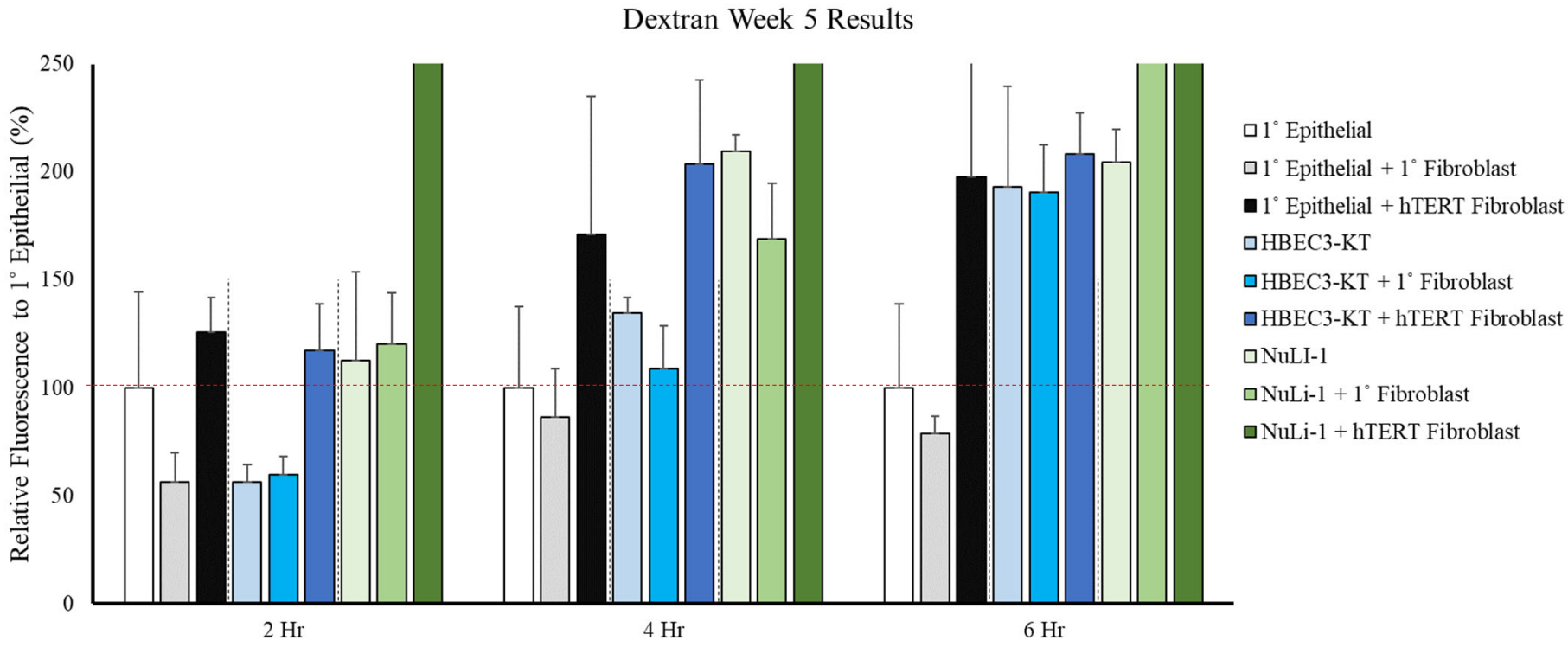
and
50% Fibroblast Basal Medium (ATCC® PCS-201-030™)
Supplemented with Fibroblast Growth Kit-Low serum (ATCC® PCS-201-041™)



Airway model characterization : dextran transportation

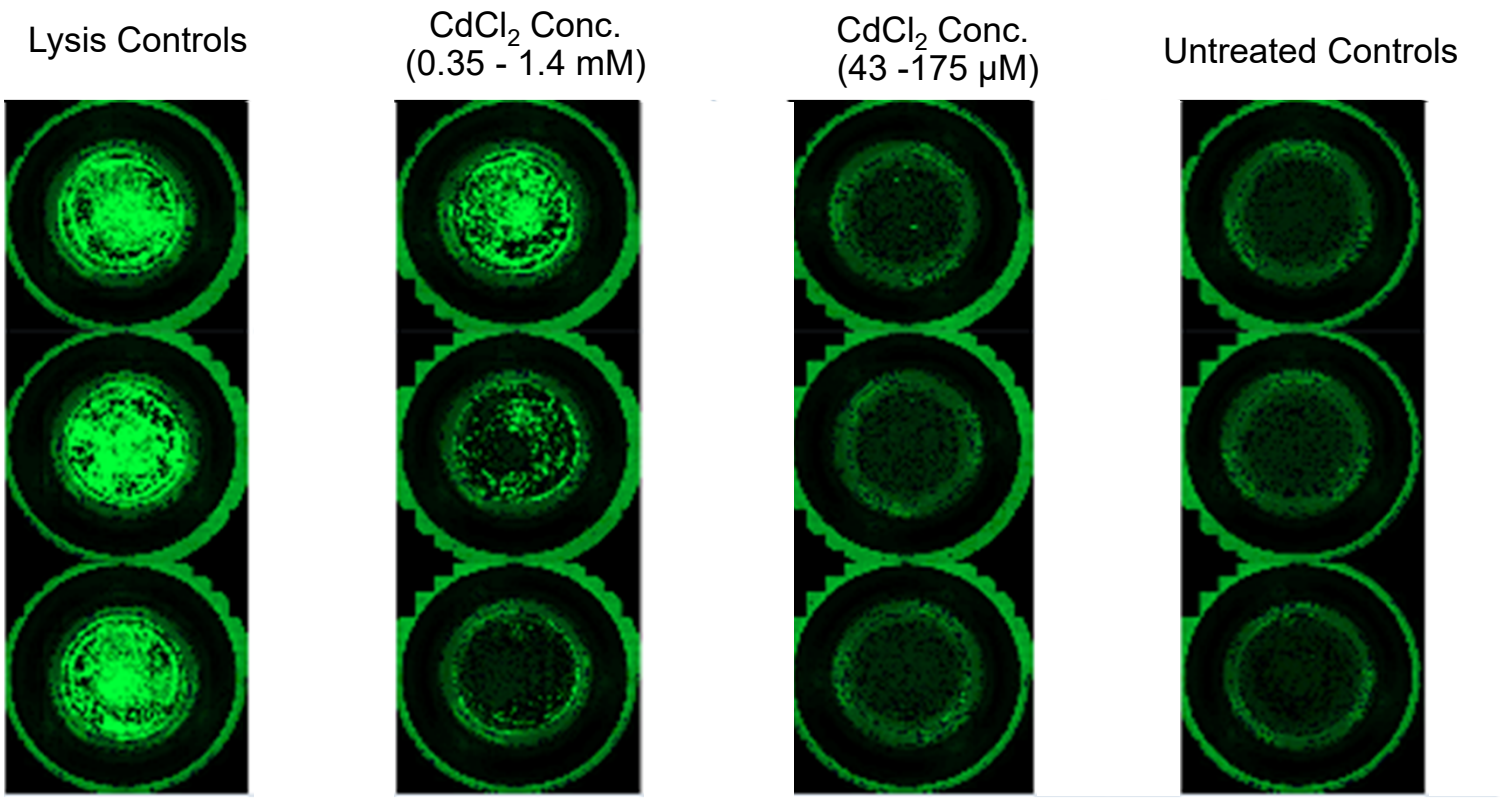


Airway model cytotoxicity

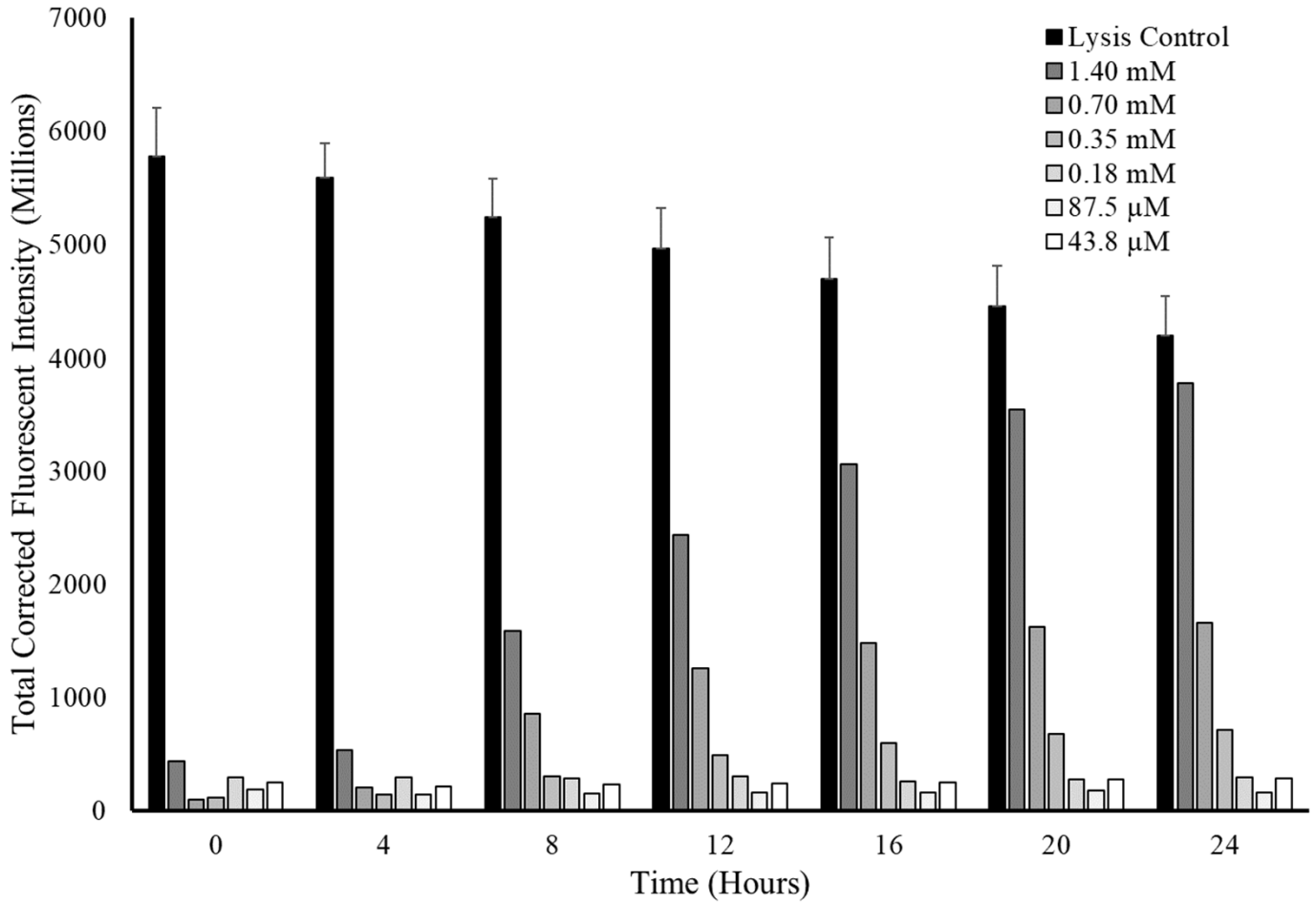
- Initial preliminary toxicology testing assessed airway model response to cadmium chloride ($CdCl_2$) exposure. $CdCl_2$ was chosen due to its chemical stability and low vapor pressure. The range exposure concentrations ranged from 43 µM to 1.4 mM and is based on concentrations tested in scientific literature.

- Selected $CdCl_2$ concentrations were administered to airway models, followed by immediately placing plates in a Biospa/Cytation system for real-time measurements. Measurements were conducted using commercial assay kits. In both assay kits, increased signal corresponds to increased cell death.

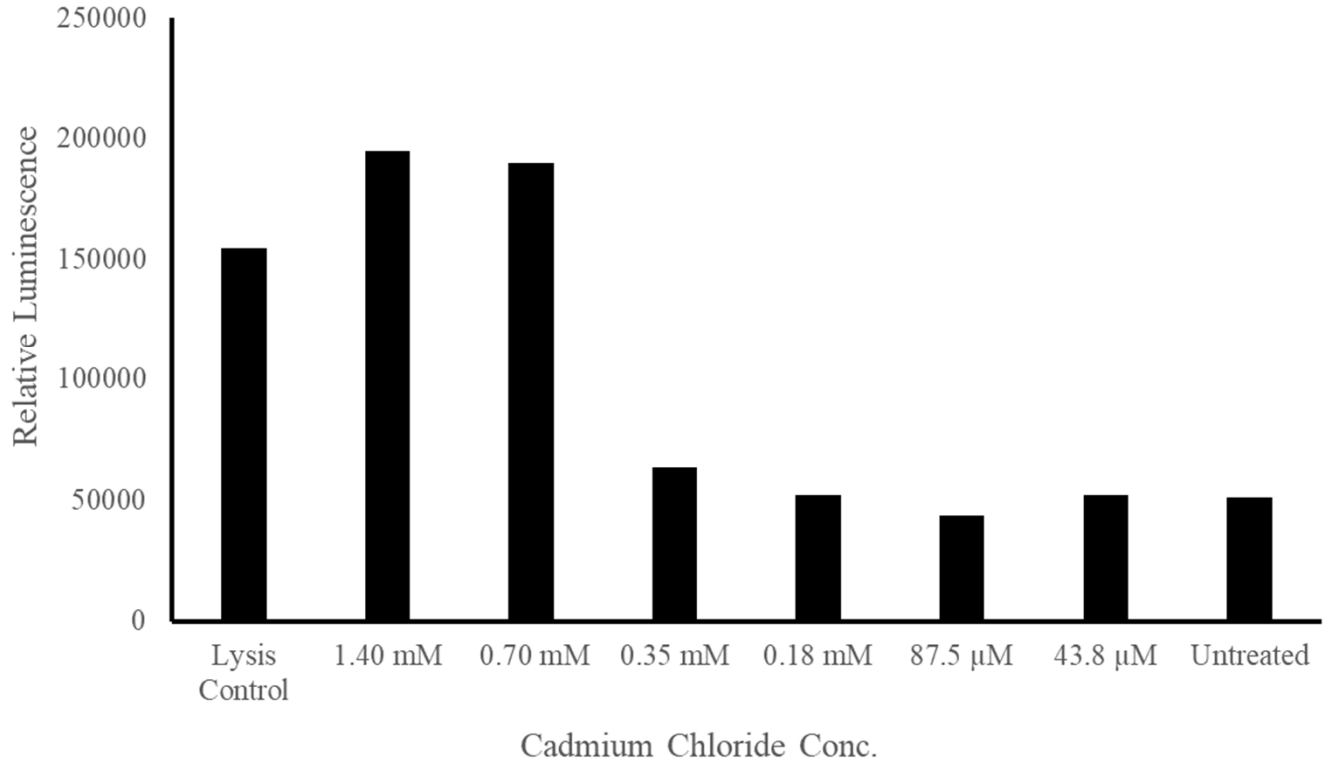
Preliminary results: CellTox™ Green Cytotoxicity assay



Preliminary results: CellTox™ Green Cytotoxicity assay

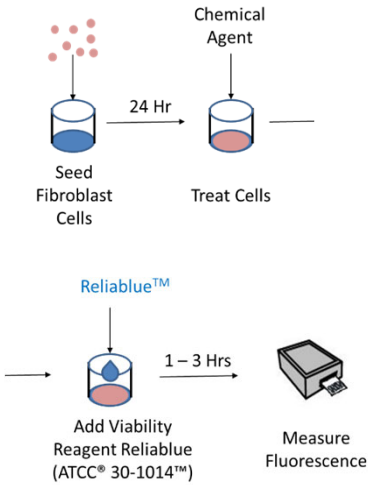


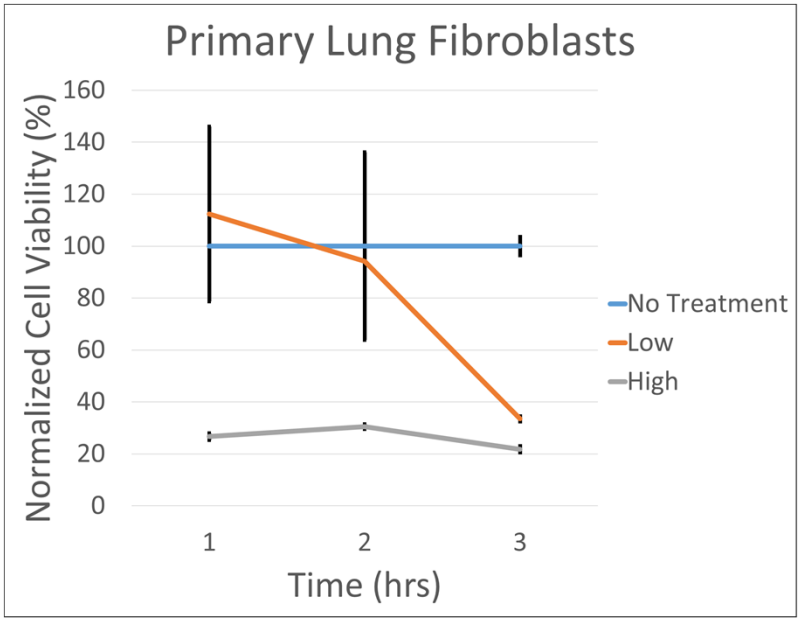
Preliminary results: LDH-Glo™ Cytotoxicity assay

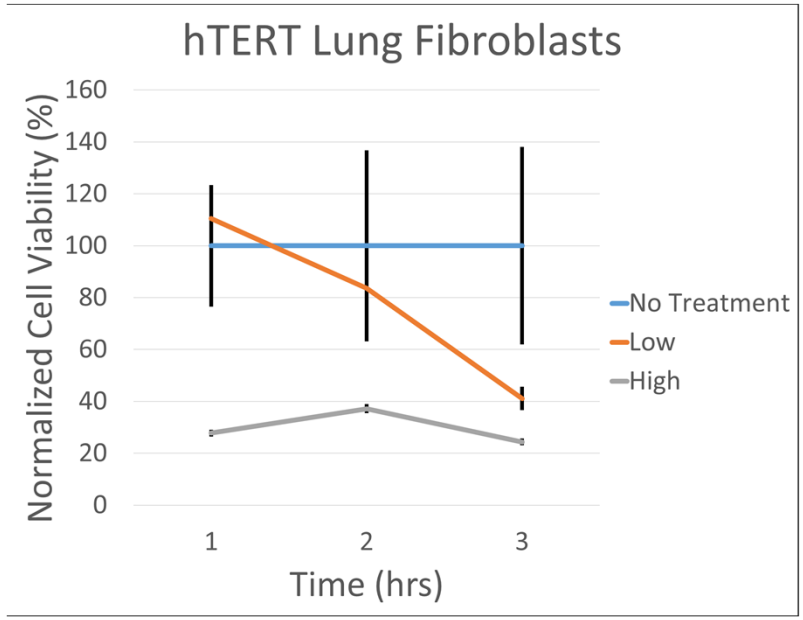


hTERT lung fibroblasts respond to chlorhexidine

Cellular cytotoxicity of lung fibroblasts by chlorhexidine is dose-dependent







Summary of airway modeling

- Airway models comprised of bronchial epithelial and fibroblasts from hTERT and primary cell types were successfully constructed.
- Histological imaging demonstrated that long-term ALI culturing resulted in the maturation of models via the presence of differentiated bronchial epithelial cells.
- TEER measurements showed that: differences exist between models comprised of primary and hTERT epithelial cells, fibroblast incorporation does not affect TEER readings, and altering media ratios made no difference in changing TEER values.
- Dextran transportation results were inversely proportional to TEER measurements.
- Preliminary toxicity testing showed that initial airway models and hTERT fibroblasts provide dose-dependent response to $CdCl_2$ ad chlorohexidine exposure, respectively. Studies are currently underway to assess toxic response from mature airway models.

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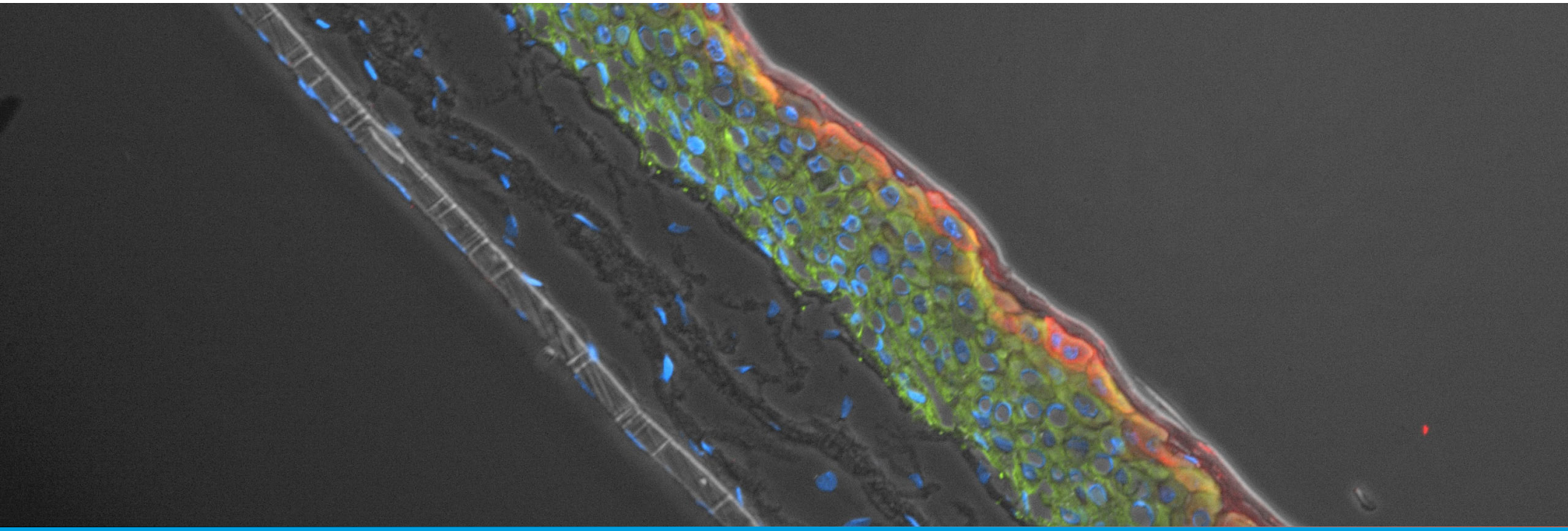

Dermal Models and Functionality

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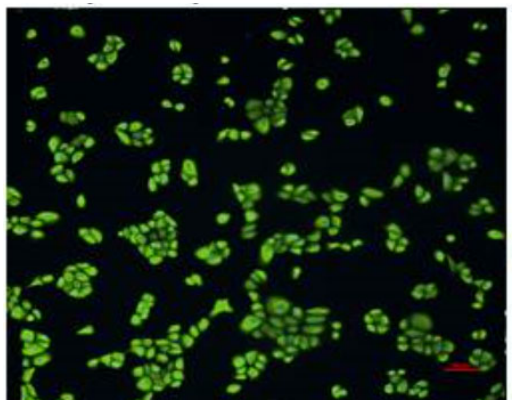
Skin models

- Primary epidermal keratinocytes
- Primary melanocytes
- Primary dermal fibroblasts

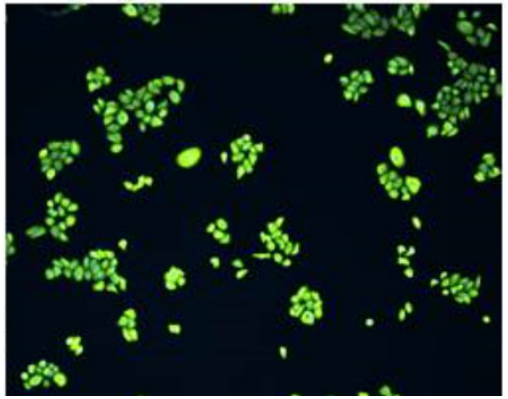
- Ker-CT (Epidermal keratinocytes)
- hTERT-immortalized Dermal Melanocyte
- BJ-5ta (Skin fibroblasts)

Ker-CT



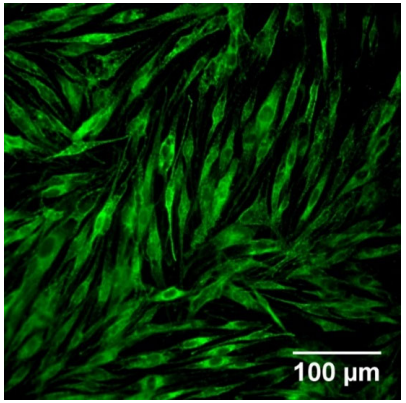
KRT5(FITC) + DAPI

Primary Epidermal Keratinocytes



KRT5(FITC) + DAPI

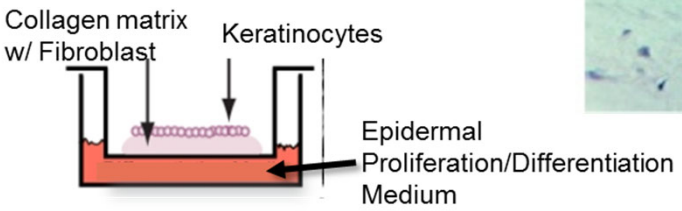
hTERT Melanocytes

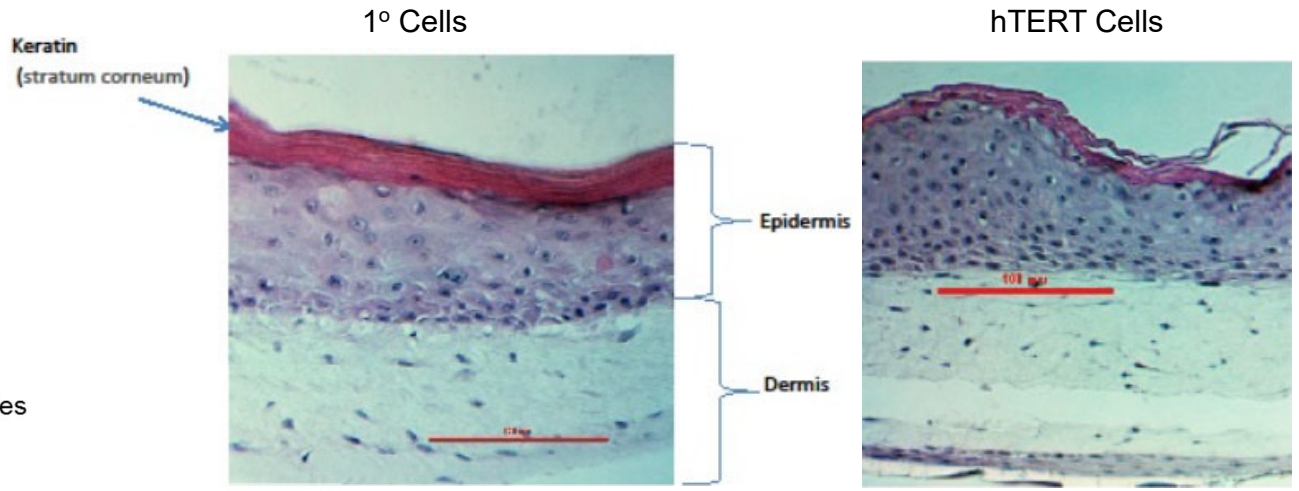


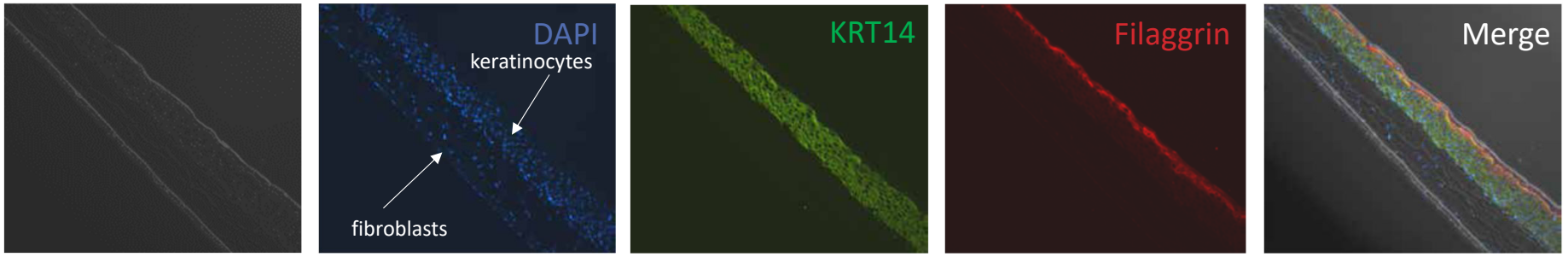
hTERT keratinocytes and hTERT fibroblasts

- Keratinocytes and fibroblast can form 3D organotypic skin cultures *in vitro*

- hTERT 3D organotypic skin cultures are positive for dermal skin markers





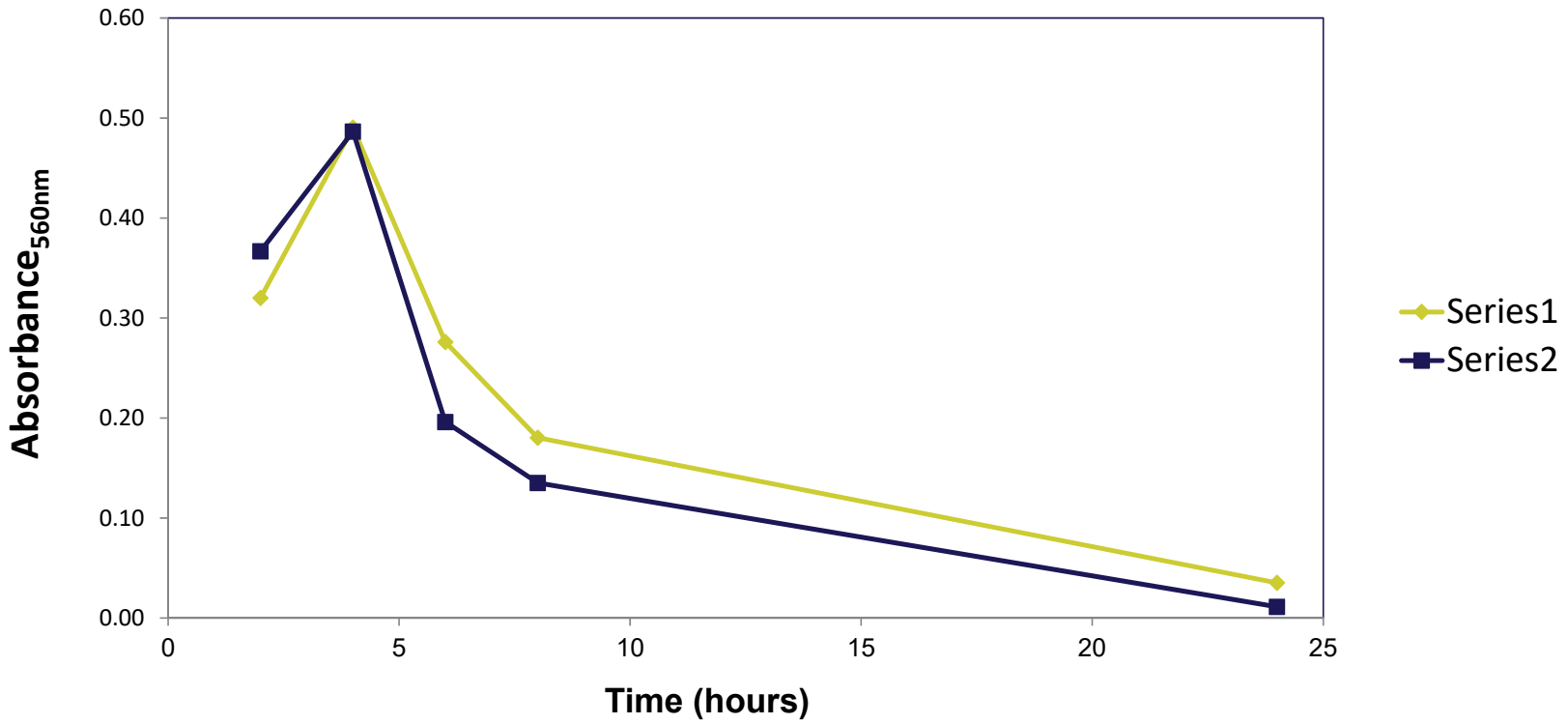


Phase contrast

Stratum granulosum/corneum

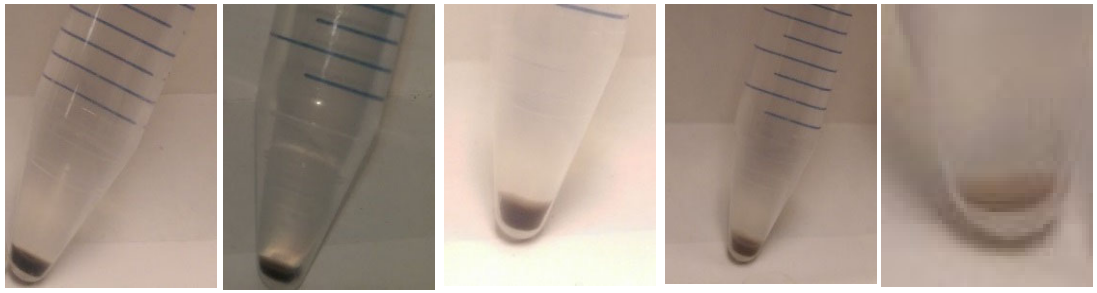
Keratinocyte 3D skin model of toxicity

3D organotypic skin culture in presence of Triton X-100. Viability monitored via MTT Assay (ATCC® 30-1010K™)



hTERT adult melanocyte characterization

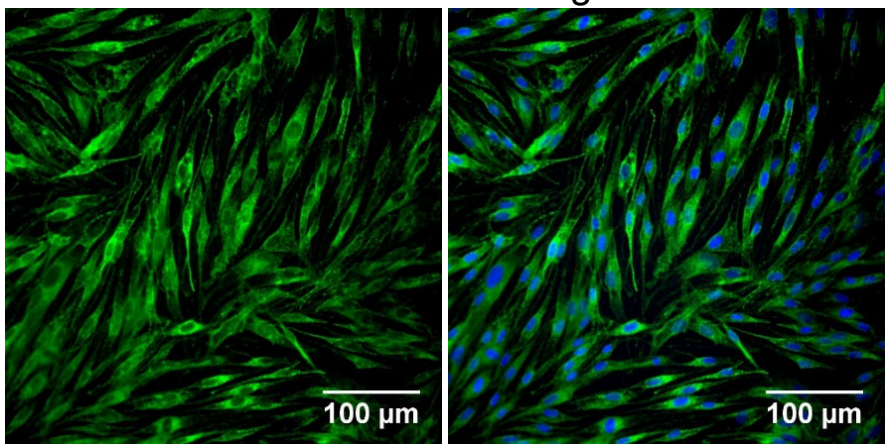
hTERT melanocytes maintain melanin production



PDL	6	15	24	40	53

hTERT melanocytes retain positive melanin marker

TYRP1 | Merged with DAPI



hTERT melanocytes lack fibroblast cell marker

TE7 | Merged with DAPI

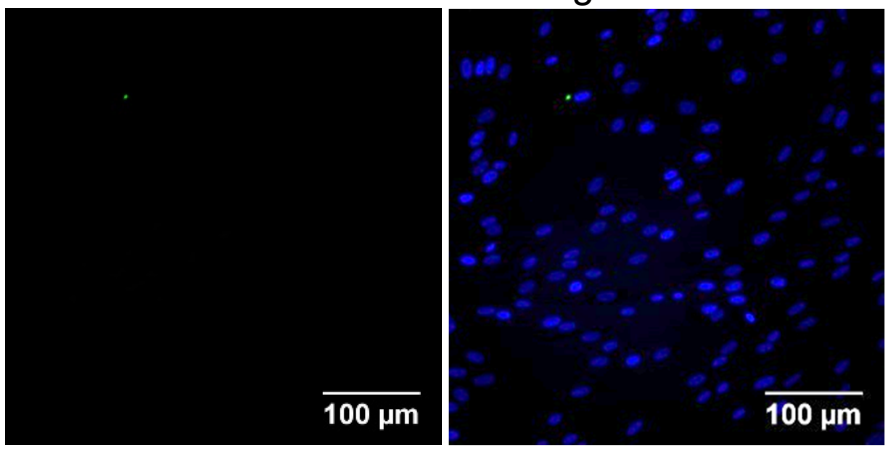
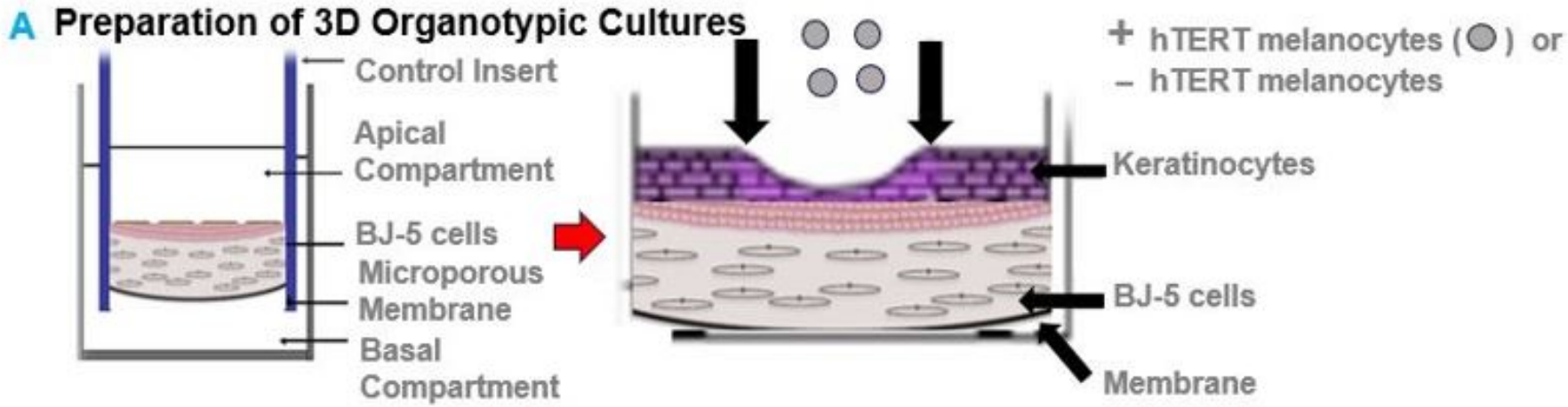


Illustration of 3D skin co-culture system

Establishing 3D Organotypic Skin Culture That Resembles Normal Skin Stratification and Pigmentation



Embed BJ-5 cells into a collagen matrix contained in a single deep well with a control insert

Condition 1. Keratinocytes / BJ-5 Fibroblasts / Neonatal Melanocytes

Condition 2. Keratinocytes / BJ-5 Fibroblasts (control)

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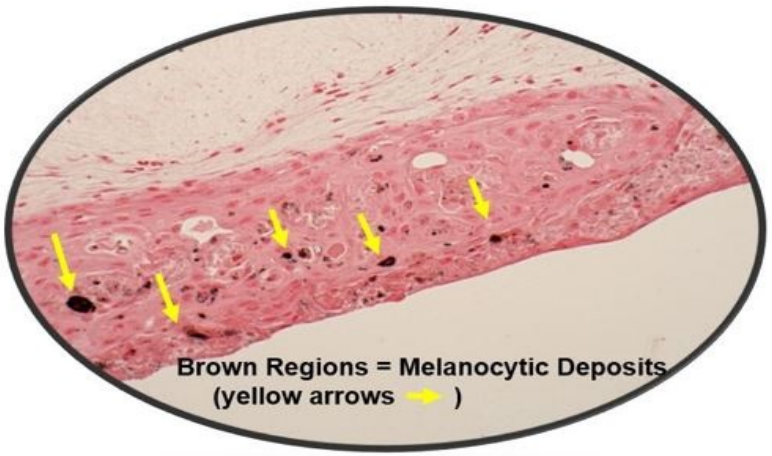
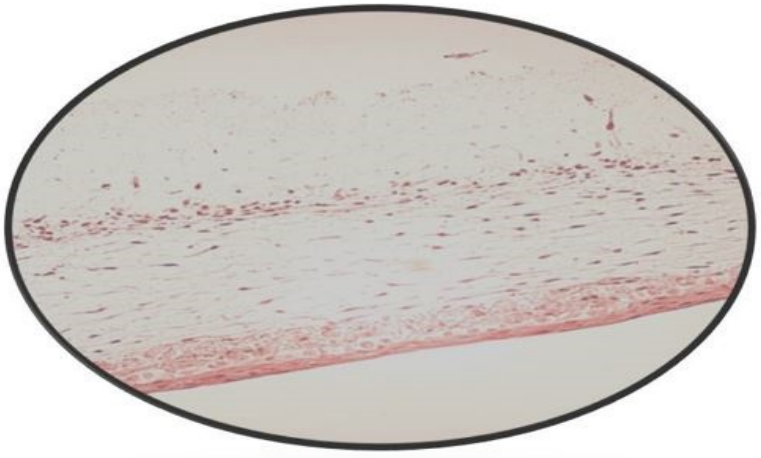
Melanin secretion in 3D co-cultured melanocytes

Fontana-Masson stain shows pigmentation from melanocytes

3D Organotypic Skin Stained for Melanin Presence



Containing hTERT Neonatal Melanocytes
(Condition 1.)

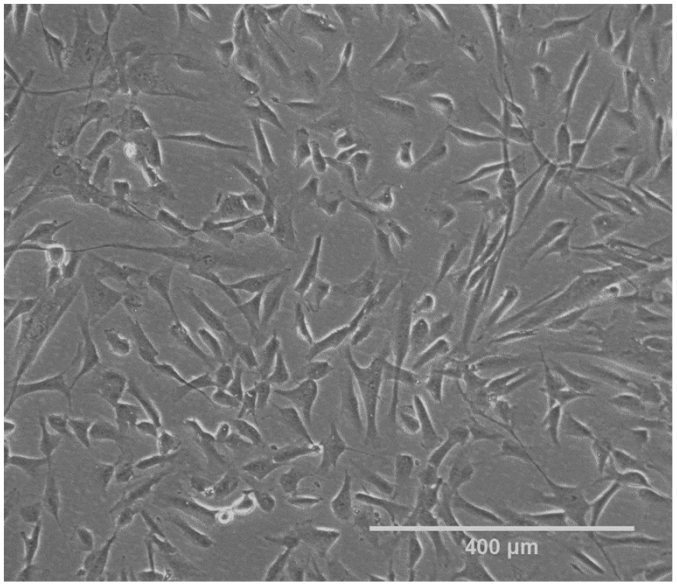


No hTERT Neonatal Melanocytes
(Condition 2.)

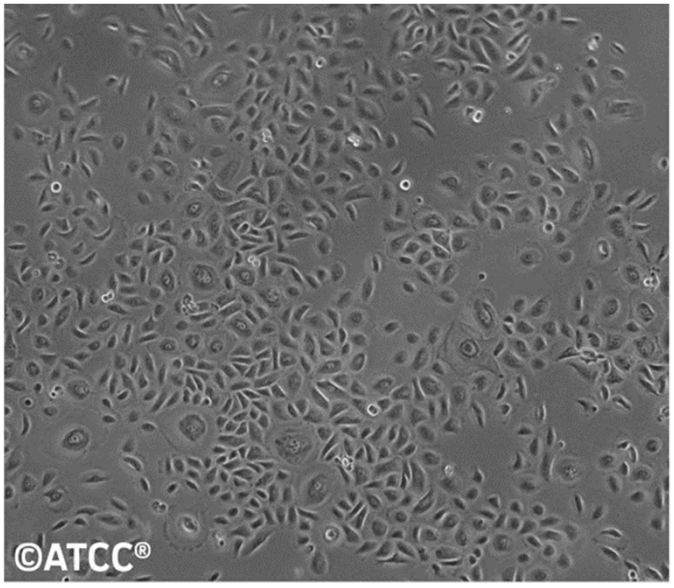
Gingival model

- Primary gingival keratinocytes
- Primary gingival fibroblasts
- hTERT gingival fibroblast

hTERT Gingival Fibroblast

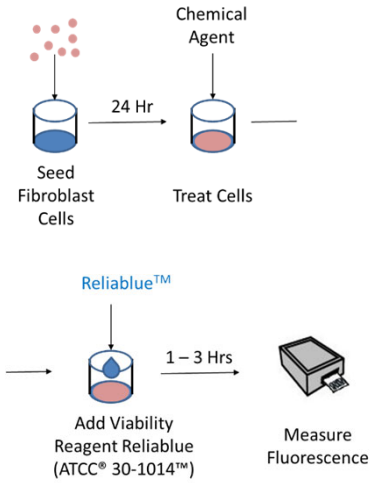


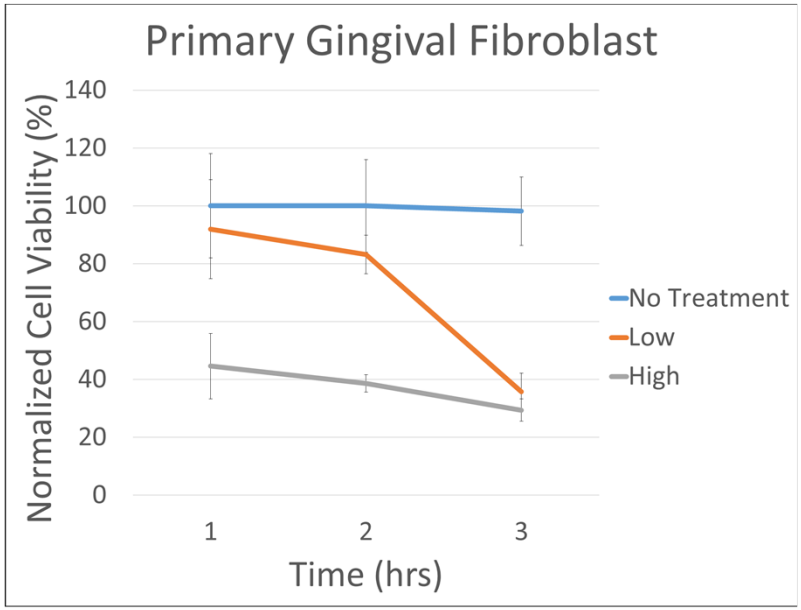
Primary Gingival Keratinocytes

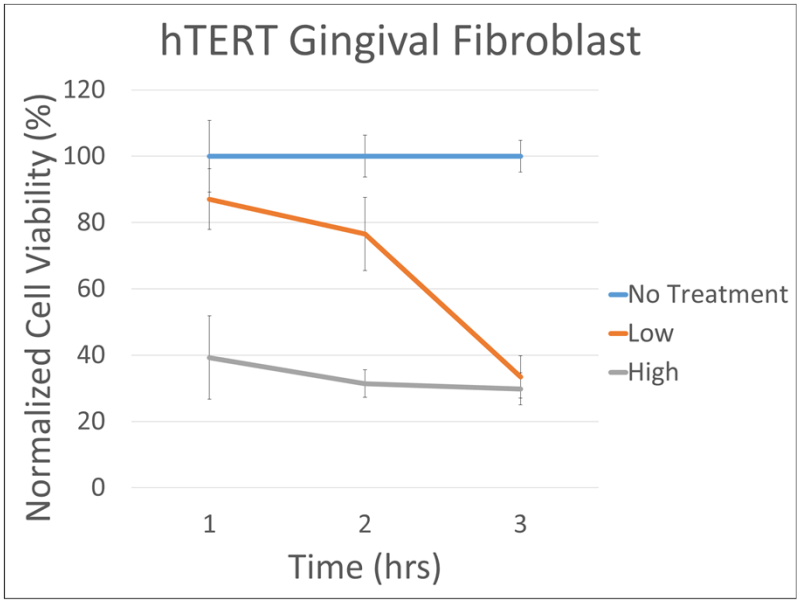


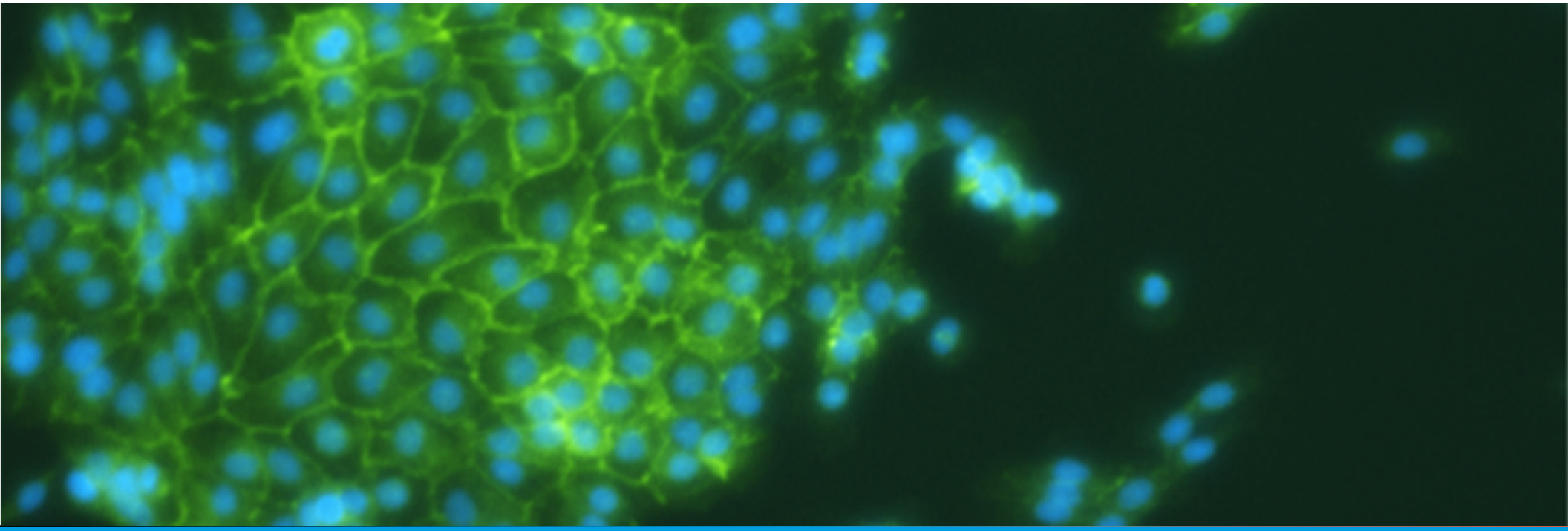
hTERT gingival fibroblasts respond to chlorhexidine

Cellular cytotoxicity of gingival fibroblast by chlorhexidine is dose-dependent









Kidney Models and Functionality

ATCC

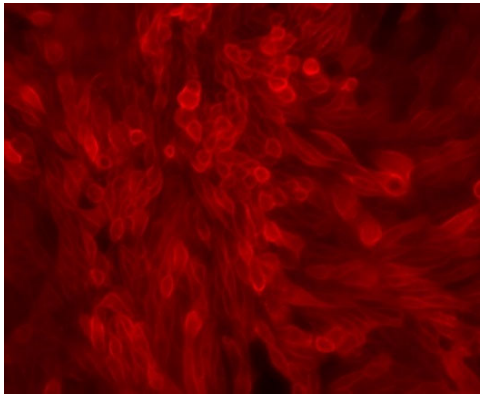
Kidney models

Renal proximal tubule epithelial cells

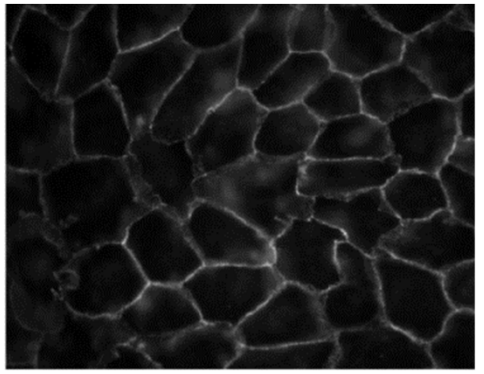
- Primary renal proximal tubule epithelial cells
- hTERT-RPTEC – immortalized renal proximal tubule epithelial cells

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

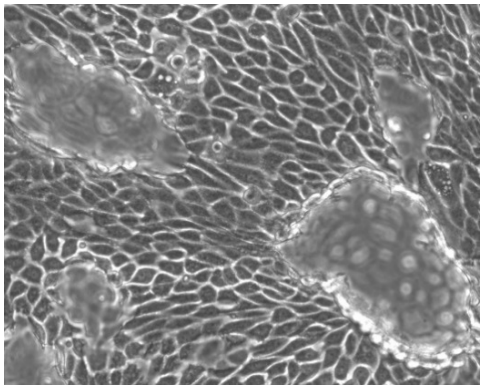
RPTEC/TERT1: CD13

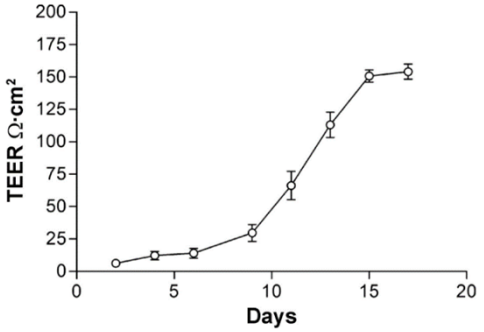


RPTEC/TERT1: E-cadherin

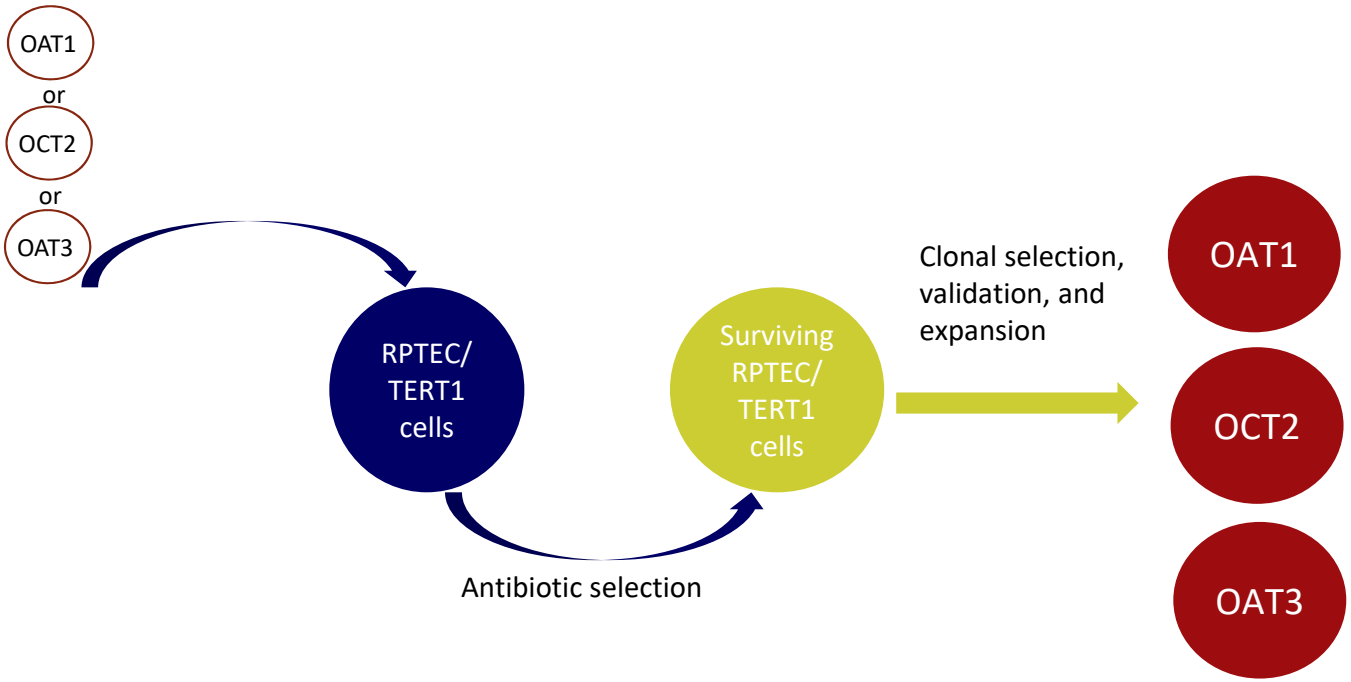


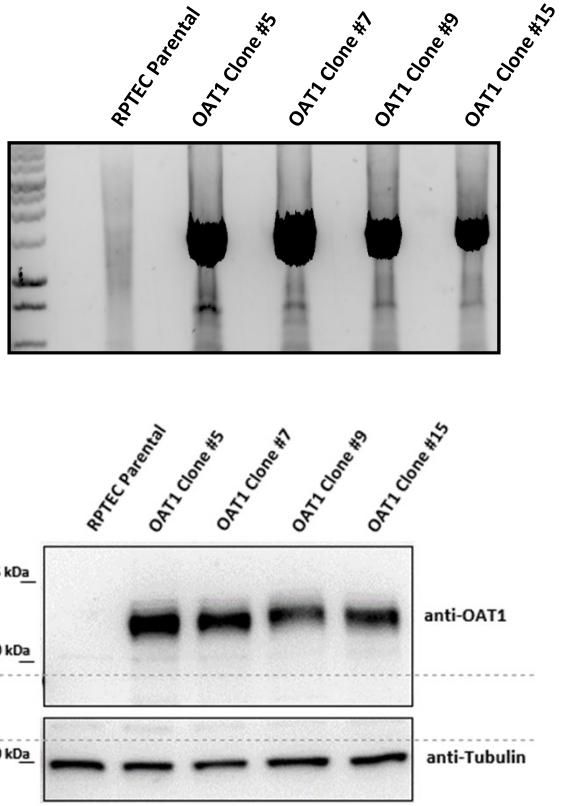
Dome formation





Enhanced kidney cellular models





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

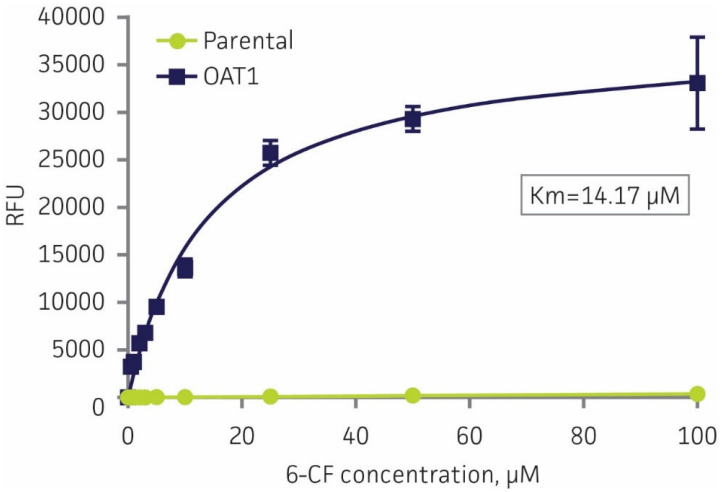
Characterized by
RT-PCR, WB, sequencing
(copy number verified)

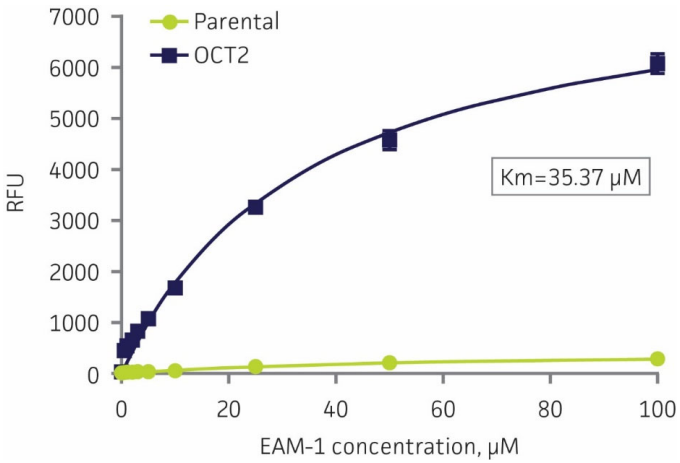
ATCC

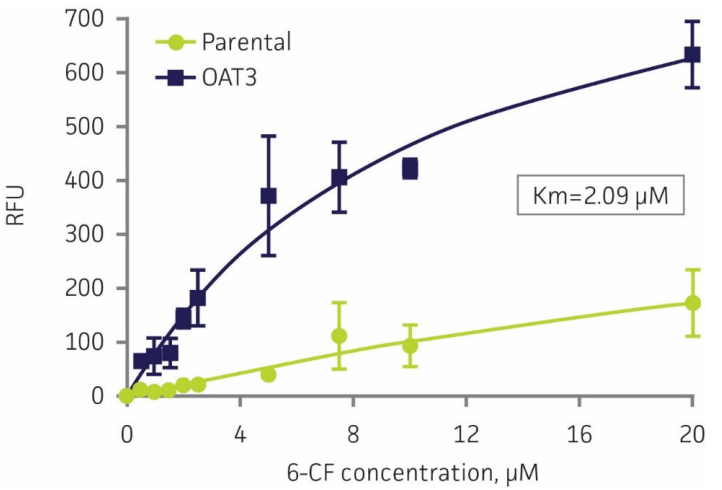
Functionality – Drug uptake assay

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





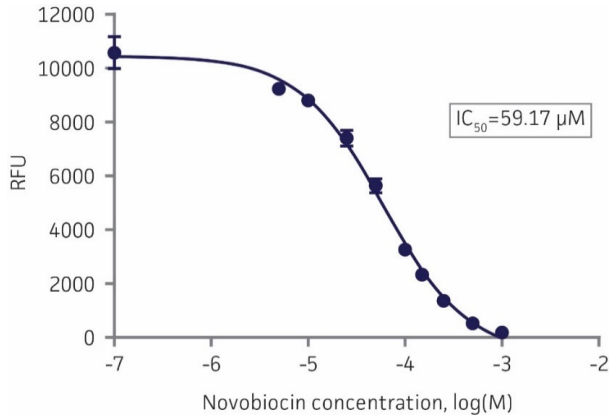


Functionality – Drug uptake inhibition assay

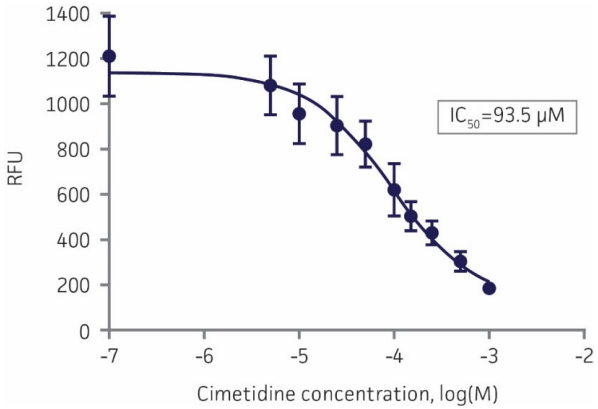
UPTAKE INHIBITION ASSAY PROTOCOL

- 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

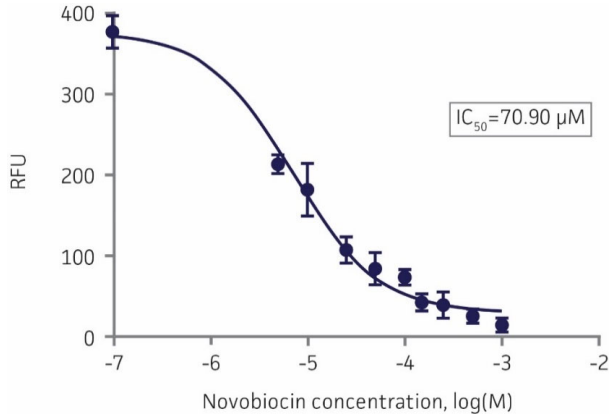
6-CF uptake inhibition in OAT-1 expressing RPTEC



EAM-1 uptake inhibition in OCT-2 expressing RPTEC



6-CF uptake inhibition in OAT-3 expressing RPTEC



Summary and resources

- ATCC is the complete solution supplier for toxicology
- From basic research through 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

Thank you and questions?

For more ATCC Toxicological Resources navigate to

www.atcc.org/TOX