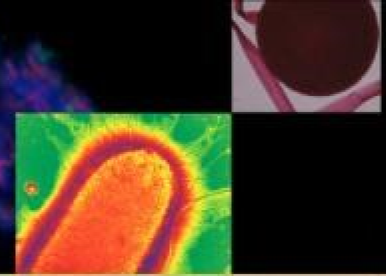
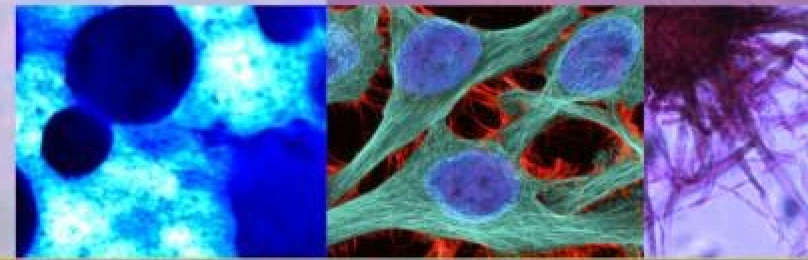
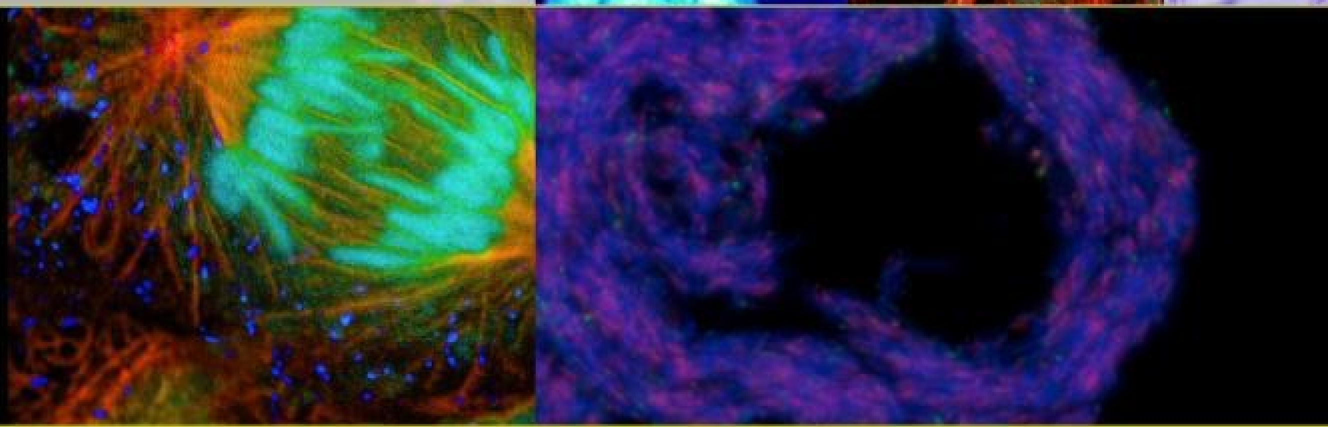
3D TISSUE MODELING



John Pulliam Ph.D.
Field Application Scientist, ATCC
November 13, 2014

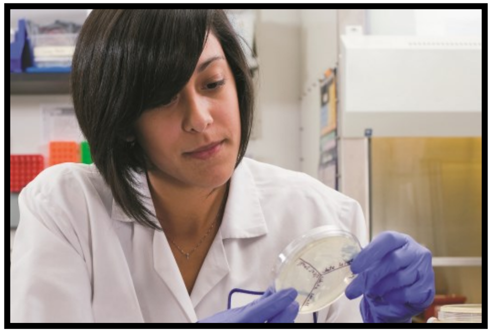




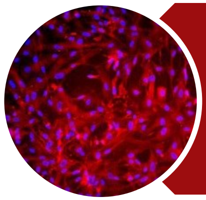
ATCC®
THE ESSENTIALS OF LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™

About ATCC

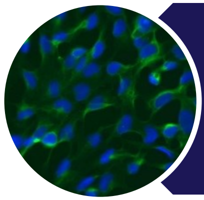
- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, fungi, yeasts, protists, and viruses
  - Microbial and tumor cell panels
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents



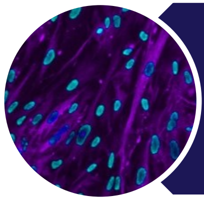
Outline



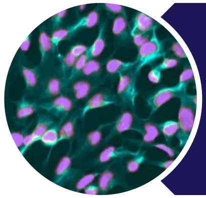
The significance of 3D culture



Air-liquid interface respiratory models

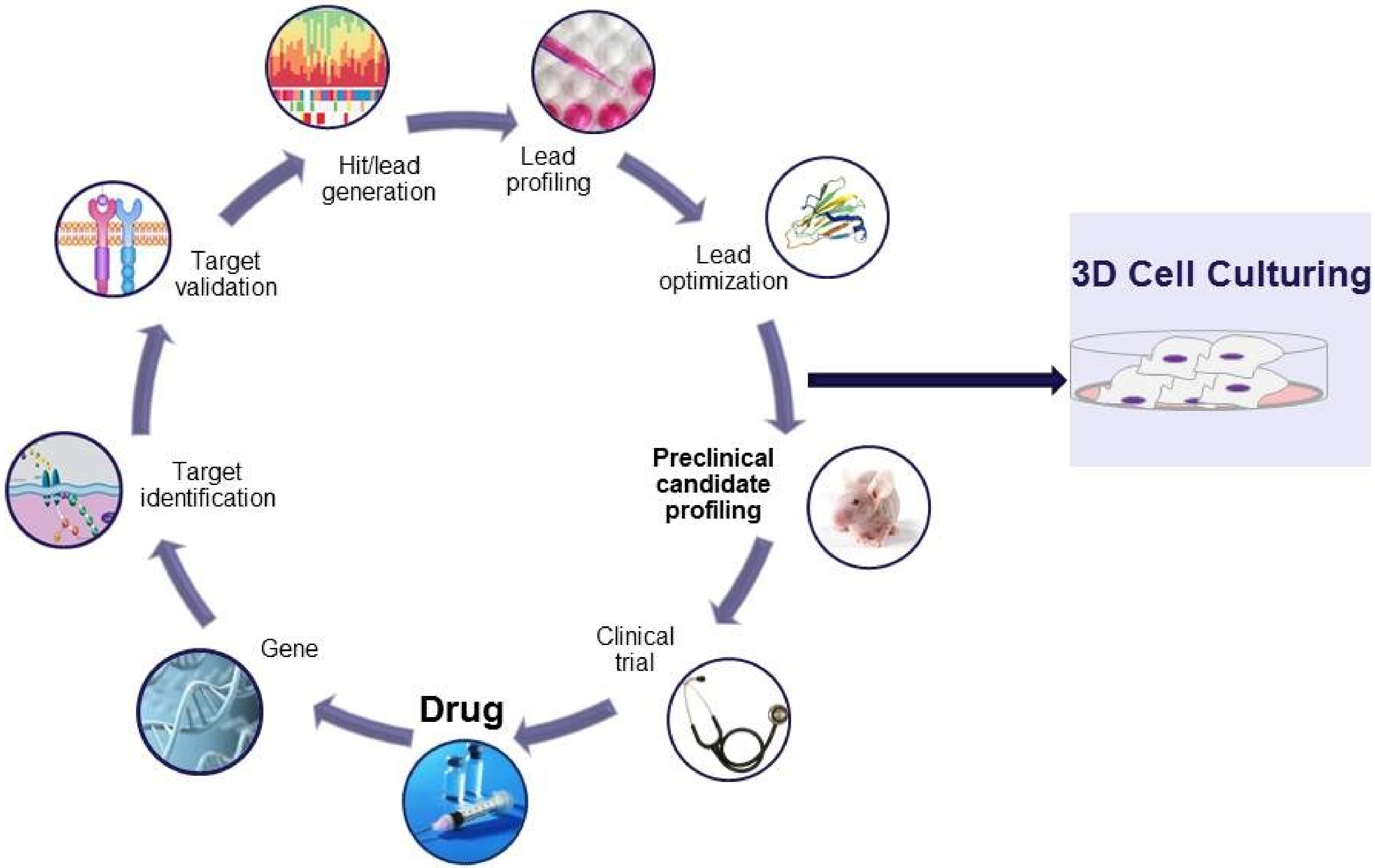


Dermatologic models



Angiogenesis models

Role of 3D culture in drug discovery



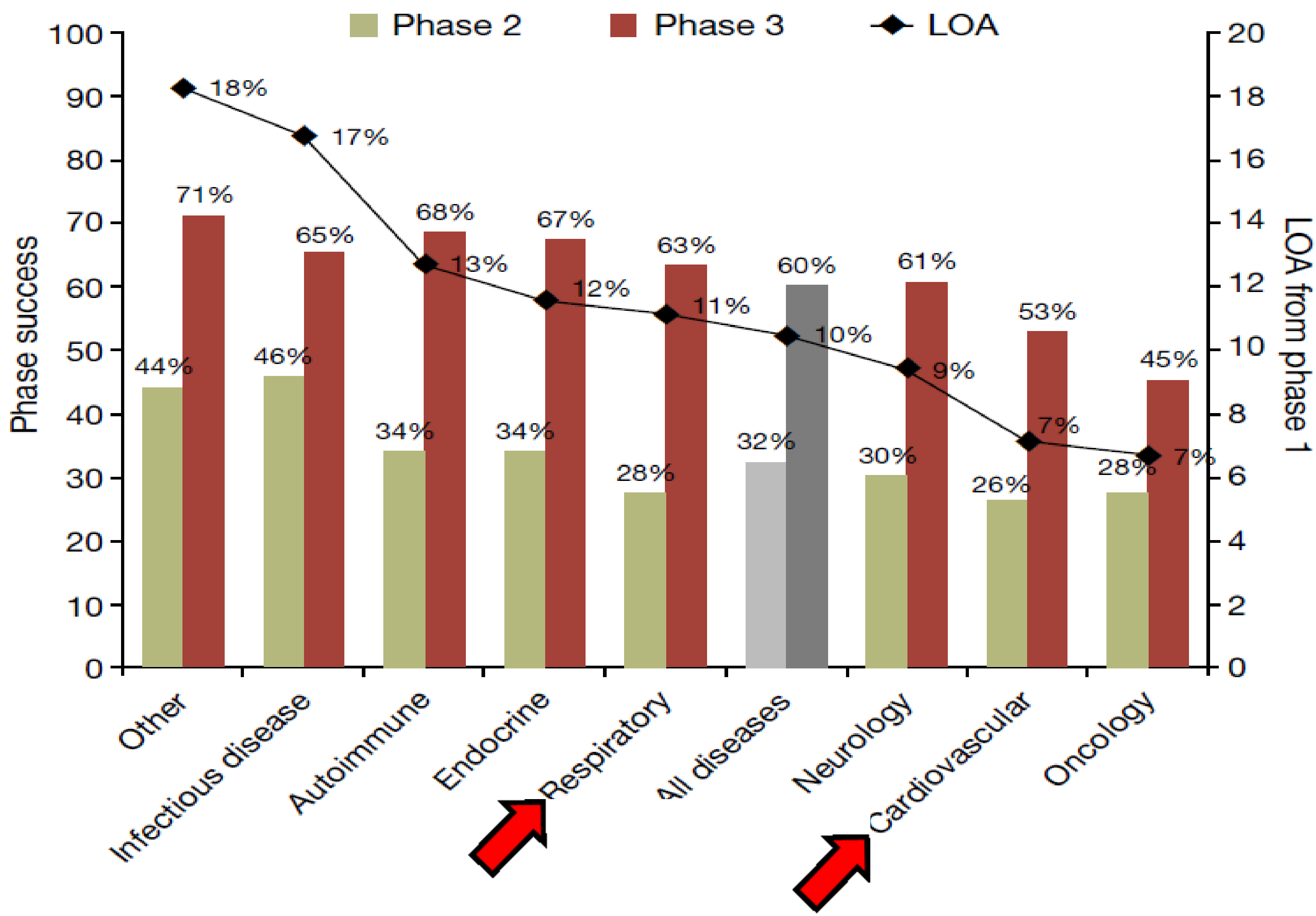
3D culture is more reflective of *in vivo* tissue conditions and may improve the predictive modeling of therapeutic drugs

Comparison of 2D and 3D culturing

Culture	Strengths	Limitations
2D	• Simplistic model • Easy to culture • Time to develop models	• Monolayer structure • Tight junctions • Non-optimal physiologic response
3D	• Complex - closer to *in vivo* tissue • Reduces need for animal models • Less cost vs animal models • Improved drug screening efficiency vs animal models	• Complexity of design • Time required to develop models

ATCC®

Likelihood of approval (LOA) by disease



Adapted from Hay *et al*. Nat Biotechnol Jan;32(1):40-51, 2014

ATCC

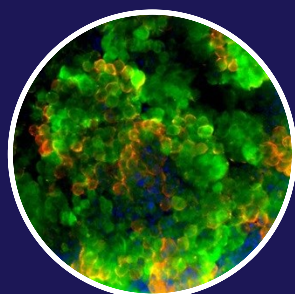
Outline

- The significance of 3D culture
- Air-liquid interface respiratory models
- Dermatologic models
- Angiogenesis models

ATCC®

ATCC Normal Human Primary Cells

- ATCC Primary Cells provide complete culture reagents formulated for optimal cell growth, morphology, and functionality
- ATCC Primary Cells are provided at very low passage

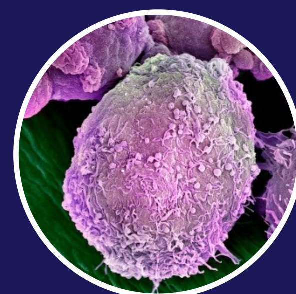


Mesenchymal

Umbilical Cord-derived

Bone Marrow-derived

Adipose-derived



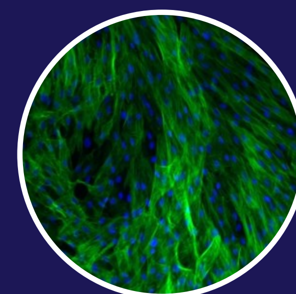
Immune

Peripheral Blood Monocytes (PBMC)

PBMC CD34+

Cord Blood CD34+

Bone Marrow CD34+



Smooth muscle

Aortic

Coronary Artery

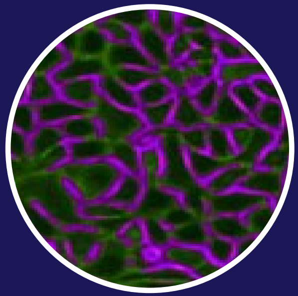
Pulmonary Artery

Lung

Bronchial/ tracheal

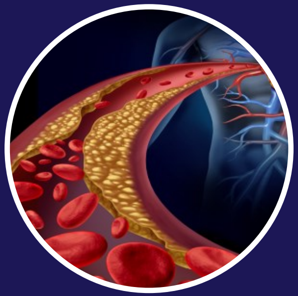
ATCC Normal Human Primary Cells

- ATCC Primary Cells provide complete culture reagents formulated for optimal cell growth, morphology, and functionality
- ATCC Primary Cells are provided at very low passage



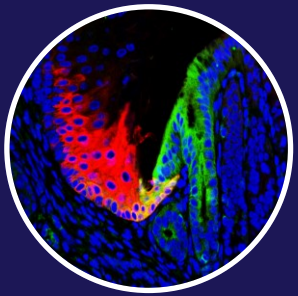
Dermal

Keratinocytes
Melanocytes
Adipose-derived
Fibroblasts



Endothelial

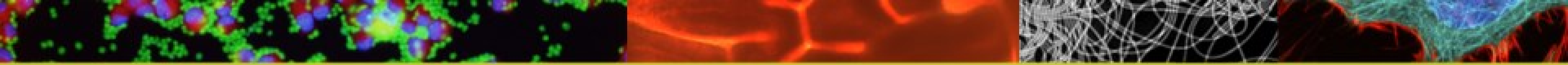
Aortic
Coronary Artery
Dermal Microvascular
Pulmonary Artery
Umbilical Vein
Umbilical Vein; Pooled



Epithelial

Bronchial/Tracheal
Small Airway
Corneal
Mammary
Prostate
Renal Proximal Tubule
Renal Distal Tubule

ATCC

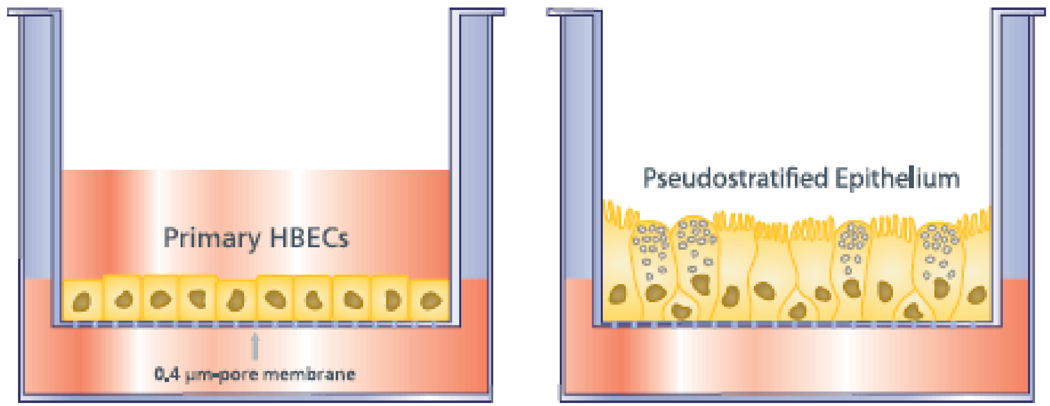


Air-liquid interface (ALI) cultures

Normal Human Small Airway Epithelial Cells (ATCC® PCS-301-010)
Normal Human Bronchial/Tracheal Epithelial Cells (ATCC® PCS-300-010)

3-4 days	2-5 days	21-28 days
Pre-expansion	**Expansion (Submerged)**	**Differentiation (Air-liquid interface)**

ATCC Primary Epithelial Cells
(used at less than passage 4)



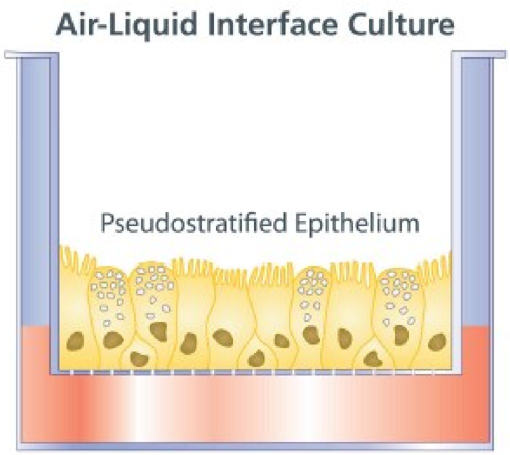
Airway Epithelial Cell Basal Medium
plus Growth Supplement Kits
(ATCC® PCS-300-030)

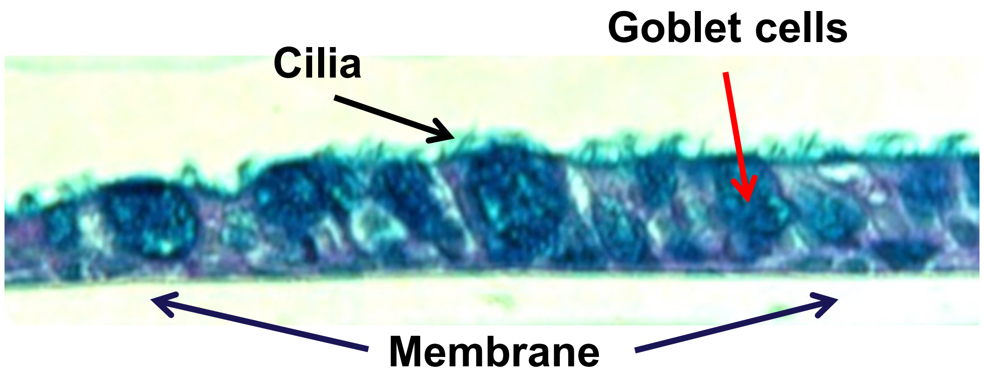
Polyethylene Terephthalate (PET) Inserts
(Corning™)

PneumaCult™ ALI Medium
(StemCell Technologies™)

http://www.veritastk.co.jp/attached/3513/29252PIS_1_1_0.ashx.pdf

Human airway epithelium





Polarized differentiated airway epithelium has the following features:

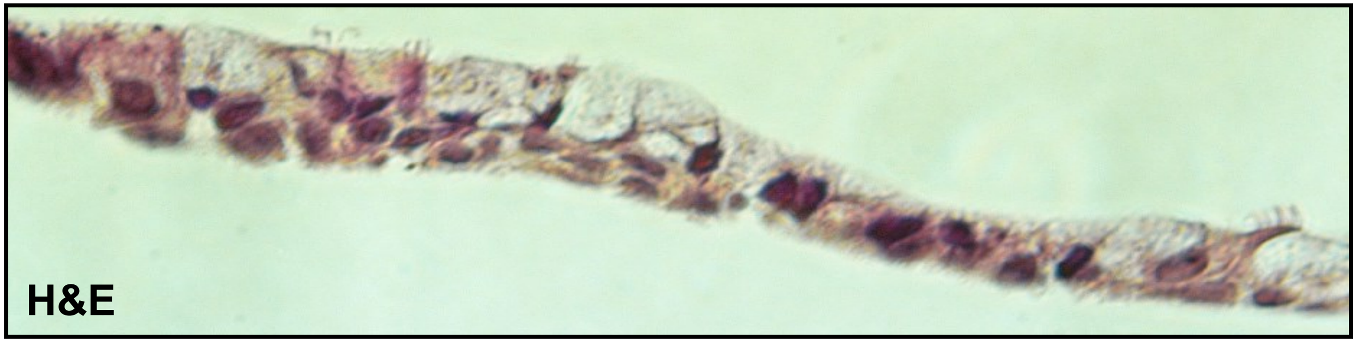
- Presence of goblet cells for mucin secretion (Periodic Acid-Schiff (PAS)-Alcian blue)
- Presence of ciliated cells (ciliogenesis)
- Presence of good barrier function (transepithelial resistance)

ATCC Human Bronchial Epithelial Cells

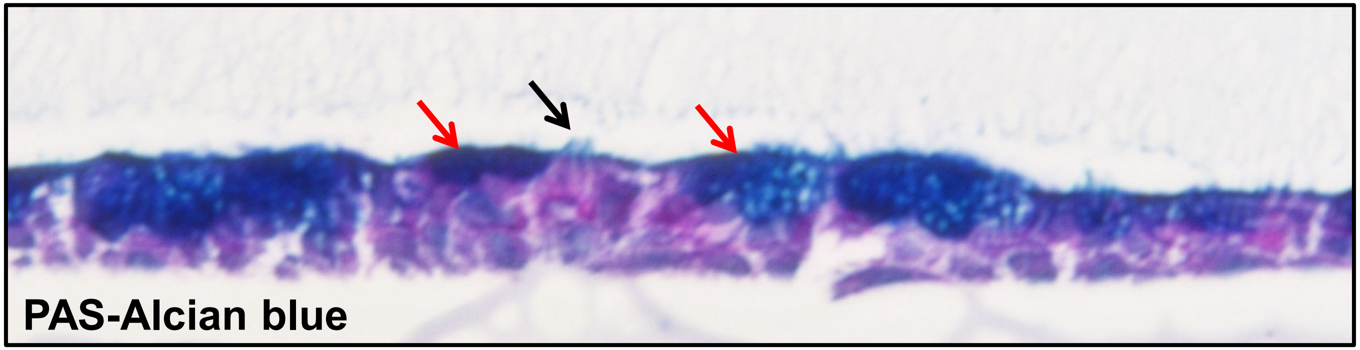
ATCC Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030) plus Bronchial Epithelial Cell Growth kit (ATCC® PCS-300-040)

At passage 3: 21 days in PneumaCult ALI differentiation media

Pseudostratified epithelium with cilia

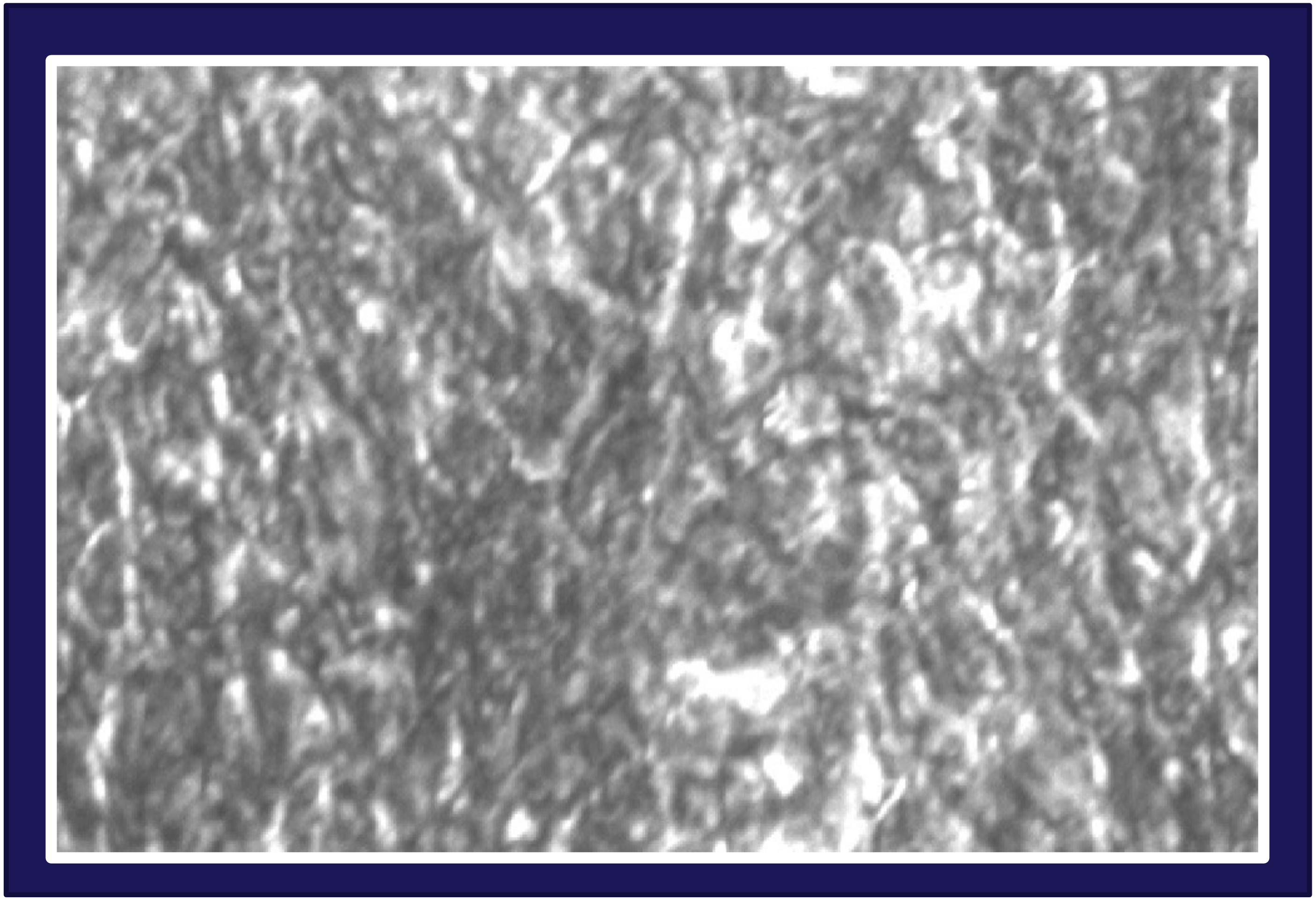


Goblet cell differentiation



Beating cilia by ALI differentiation

ATCC Human Bronchial Epithelial Cells 26 days post airlift



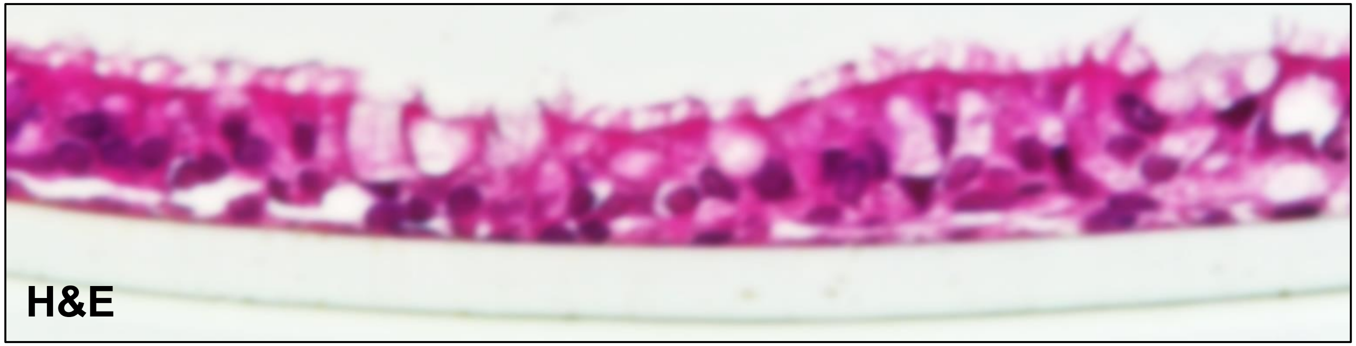
ATCC®

ATCC Human Small Airway Epithelial Cells

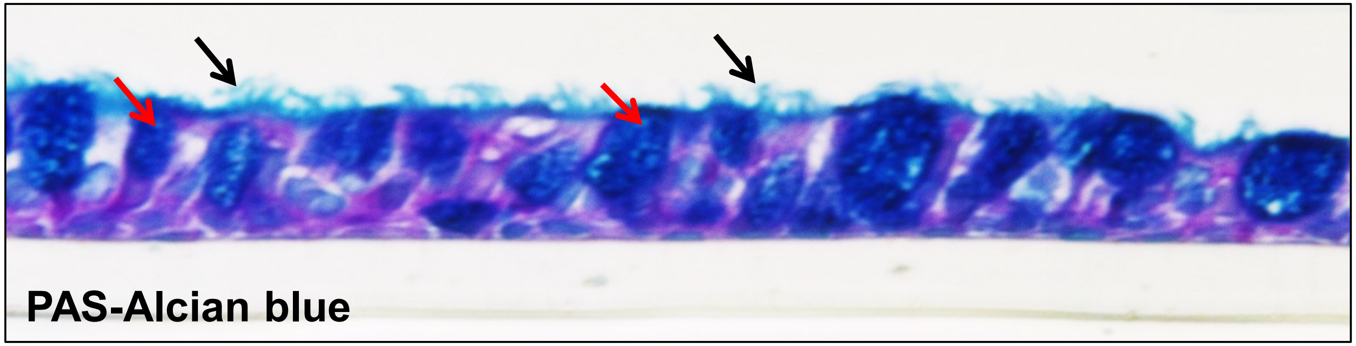
ATCC Airway Epithelial Cell Basal Medium (ATCC PCS-300-030) and Small Airway Epithelial Cell Growth kit (ATCC PCS-301-040)

At passage 3: ALI differentiation for 21 days in PneumaCult ALI differentiation media

Pseudostratified epithelium with cilia

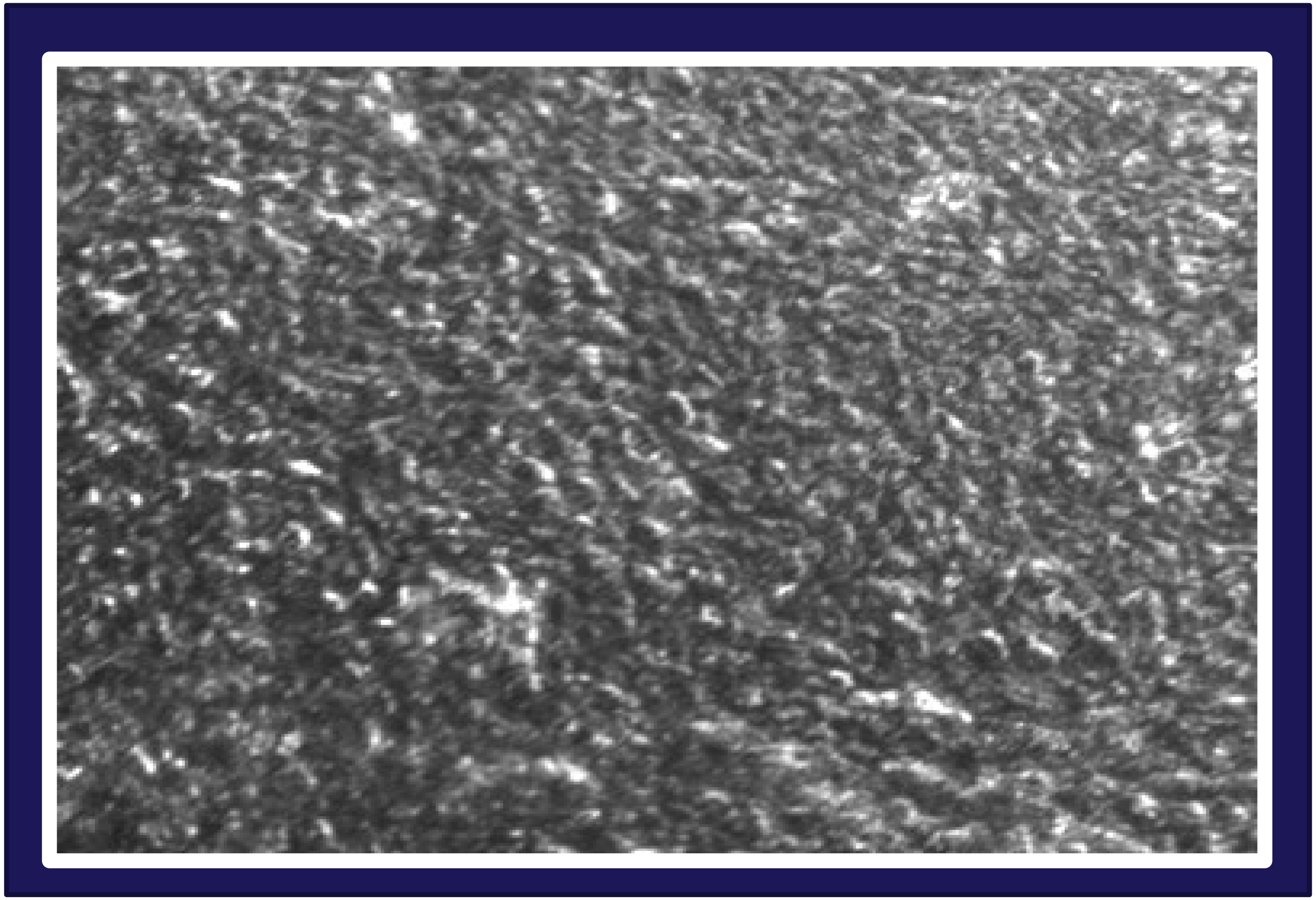


Goblet cells

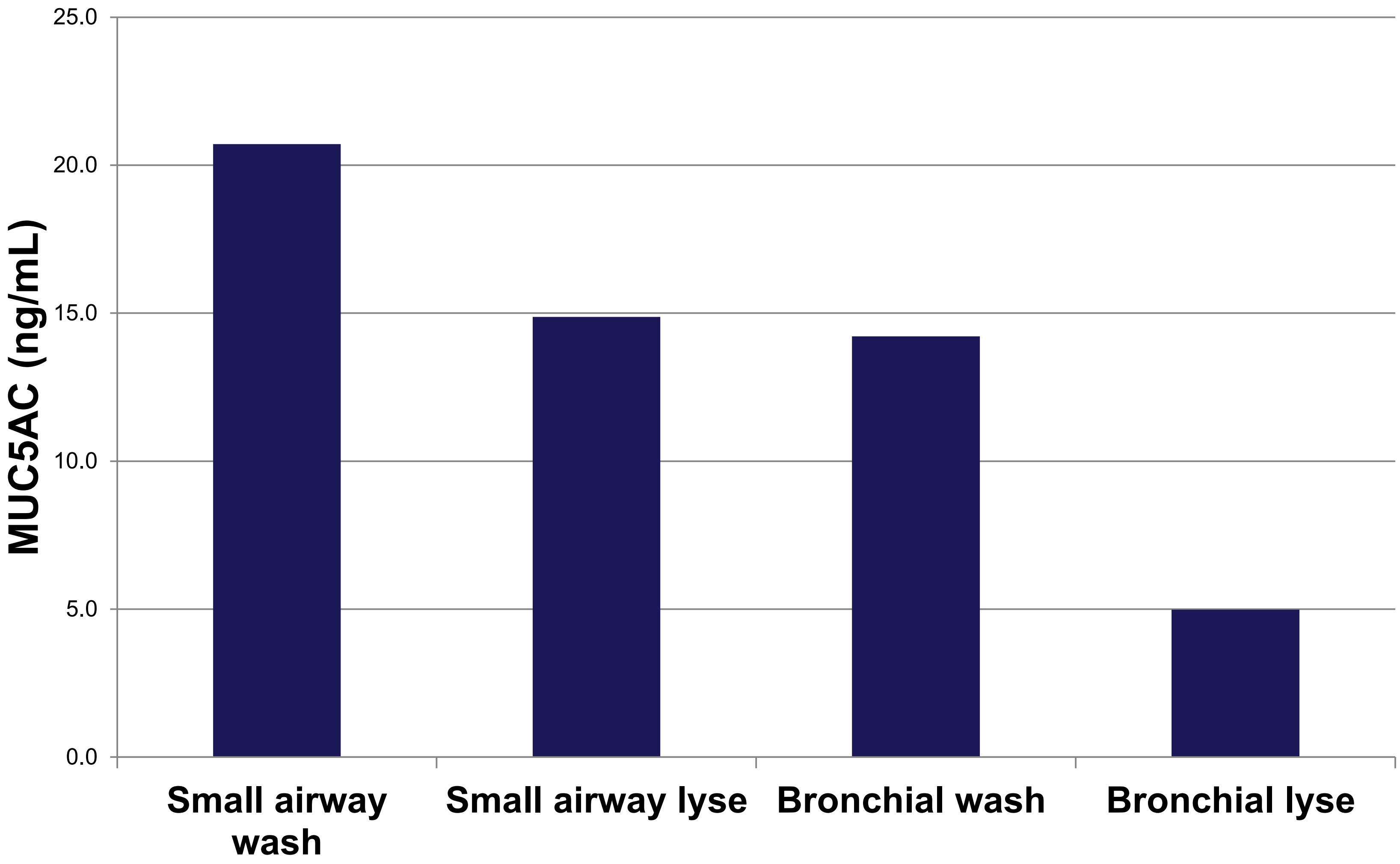


Beating cilia by ALI differentiation

ATCC Human Small Airway Epithelial Cells 25 days post airlift

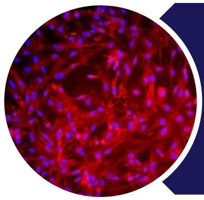


Mucin secretion, Primary Small Airway and Bronchial Epithelial Cells 28 days post airlift

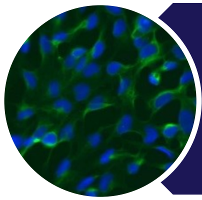


ATCC®

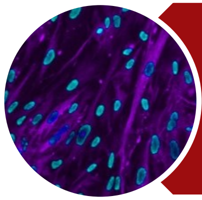
Outline



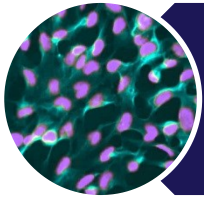
The significance of 3D culture



Air-liquid interface respiratory models



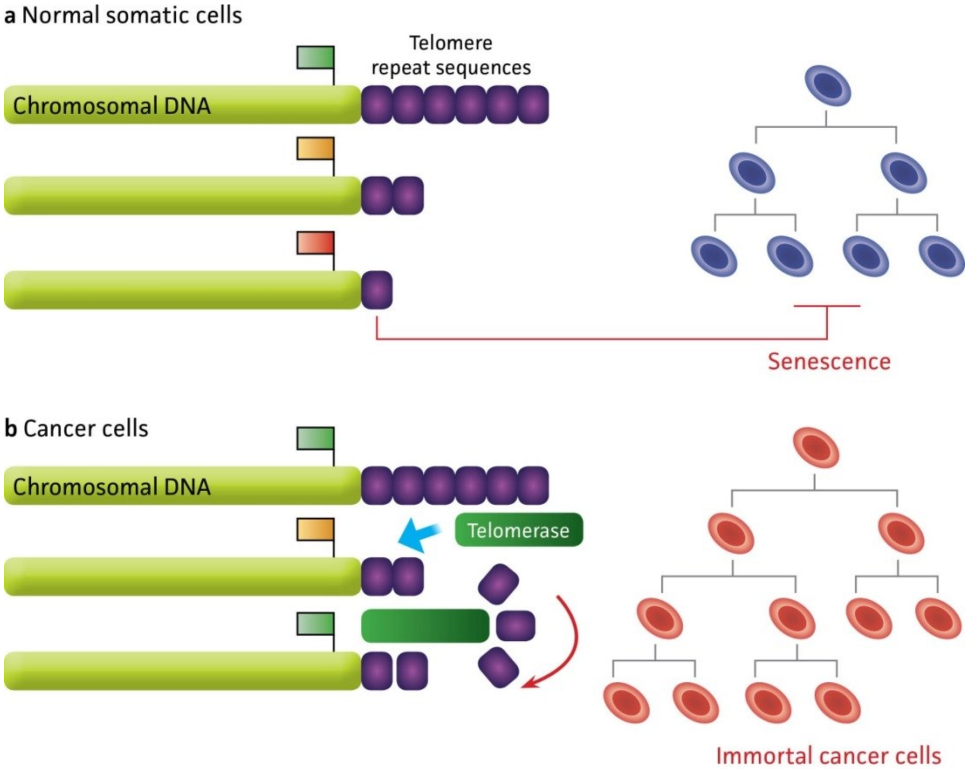
Dermatologic models



Angiogenesis models

Bypassing replicative senescence

Overexpression of telomerase and supportive oncoproteins in primary cells



Note: Viral (Large T and small T antigen, HPV-16 E6/E7) and non-viral (Cdk-4 and Bmi-1) onco-protein vectors may also be used to support the hTERT immortalization vector

Keith W *et al.* Expert Rev Mol Med 22;4(10):1-25, 2002.

hTERT Immortalized Cells - unique tools

	Primary cells	hTERT immortalized	Oncogene, viral immortalized	Cancer cell lines
Mimic *in vivo* Tissue Phenotype	++++	+++	++	+
Genotypic Stability	Diploid	Diploid / Near diploid	Near diploid / Aneuploid	Aneuploid
Proliferative Capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-experimental Consistency	Low	Good	Good	Good
Cost	High	Medium	Low	Low
Ease of Use	+	++	++	+++

Pros and cons of different cell models for tissue-relevant functional studies

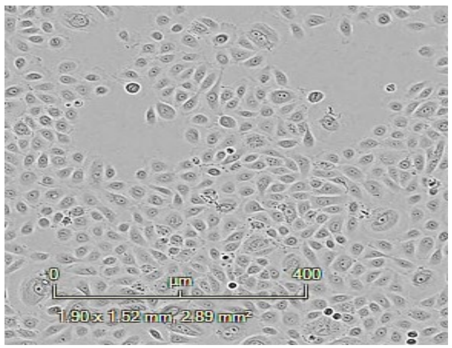
hTERT immortalized cells combine the physiological nature of primary cells and the ability to be cultured continuously, avoiding the limitations of both types while still reaping their benefits.

ATCC®

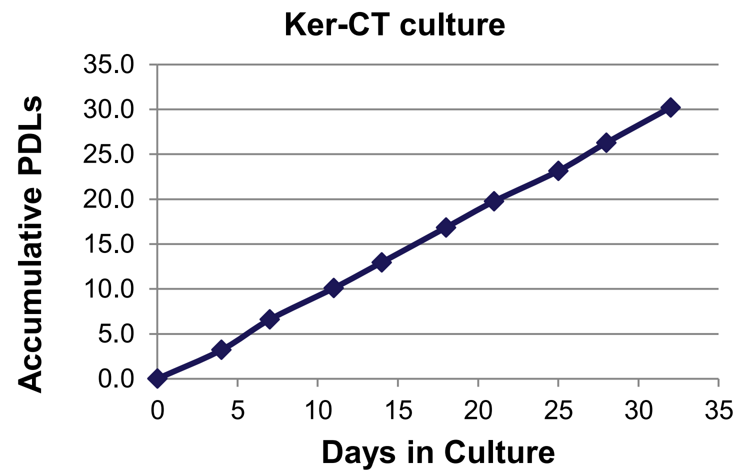
Ker-CT– Immortalized Keratinocytes retain intact differentiation capability

- Ker-CT (ATCC® CRL-4048™): immortalized by hTERT and CDK4 from neonatal foreskin keratinocytes

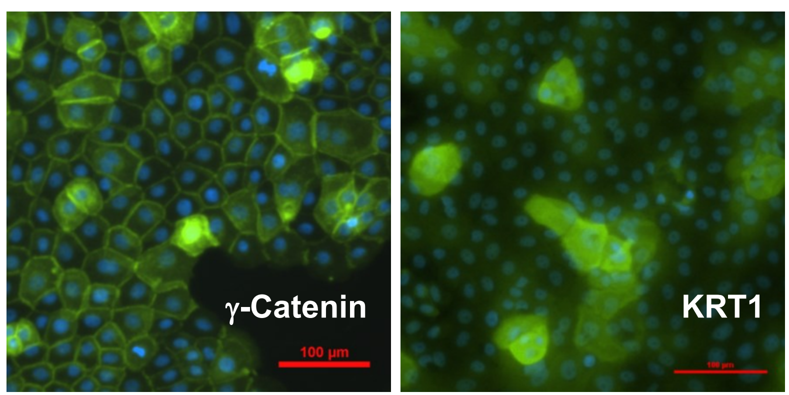
Proliferating



5000 cell/cm^2, Day 3



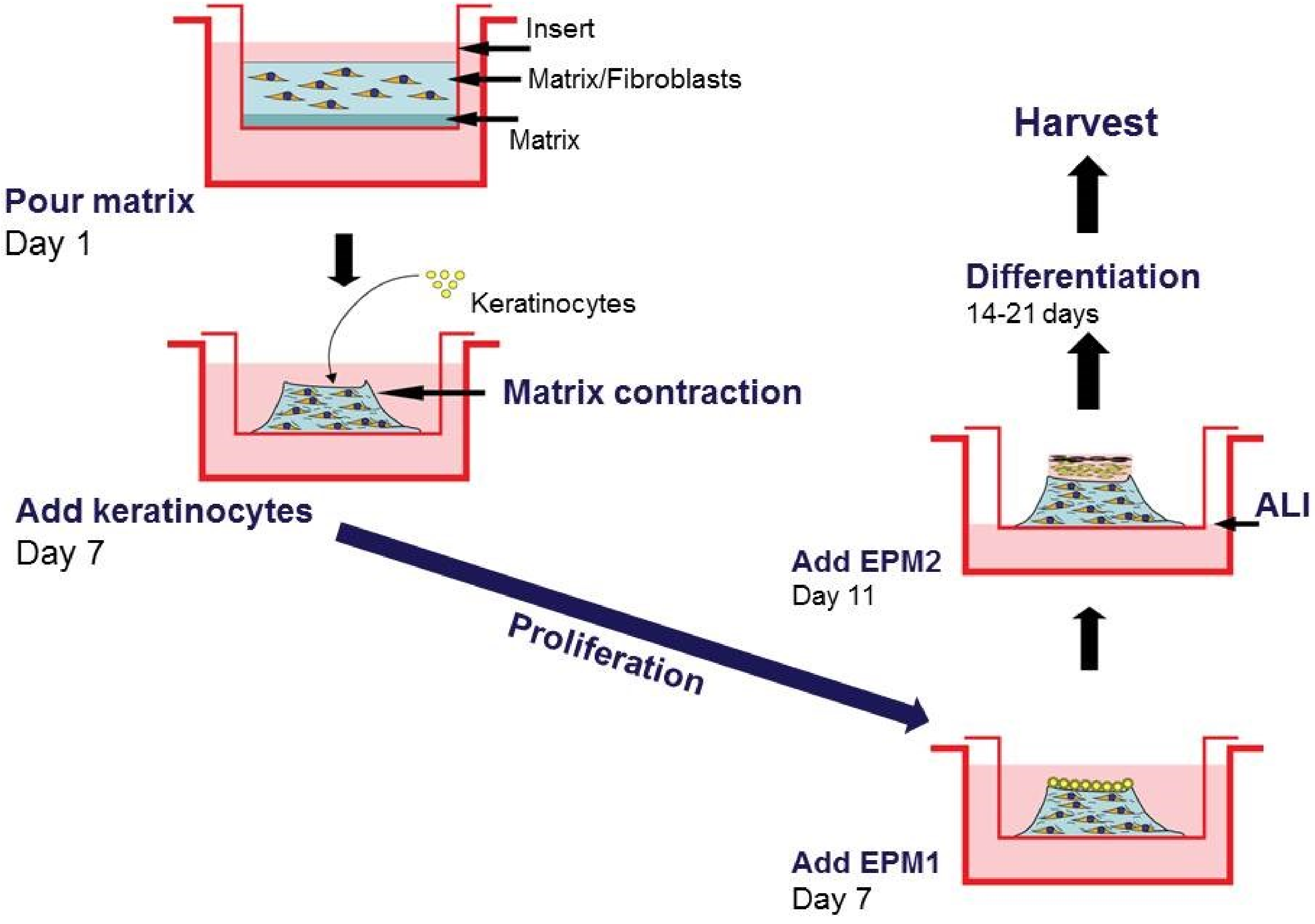
Differentiation



2D differentiation, Day 4

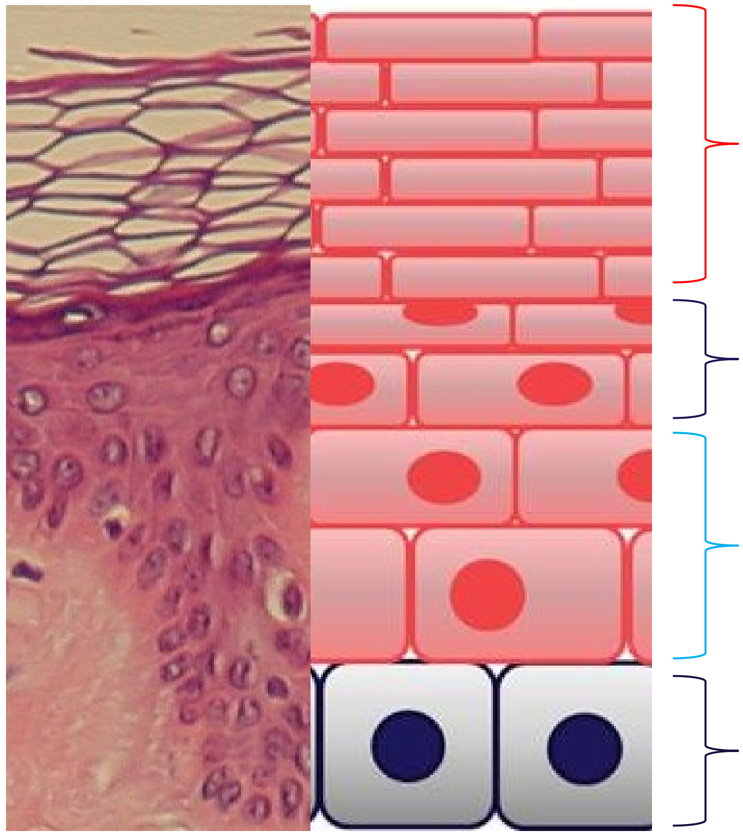
Ramirez R, *et al*. Oncogene 22(3): 433-44, 2003.

Keratinocytes grown in raft co-culture



Adapted from Kalabis J *et al*. Nature Protocols 7:235-246, 2012.

Differentiation of epidermal keratinocytes



Cornified

Granular

Spinous

Basal

Cornification
- Cornified cell envelope
- Nuclear breakdown

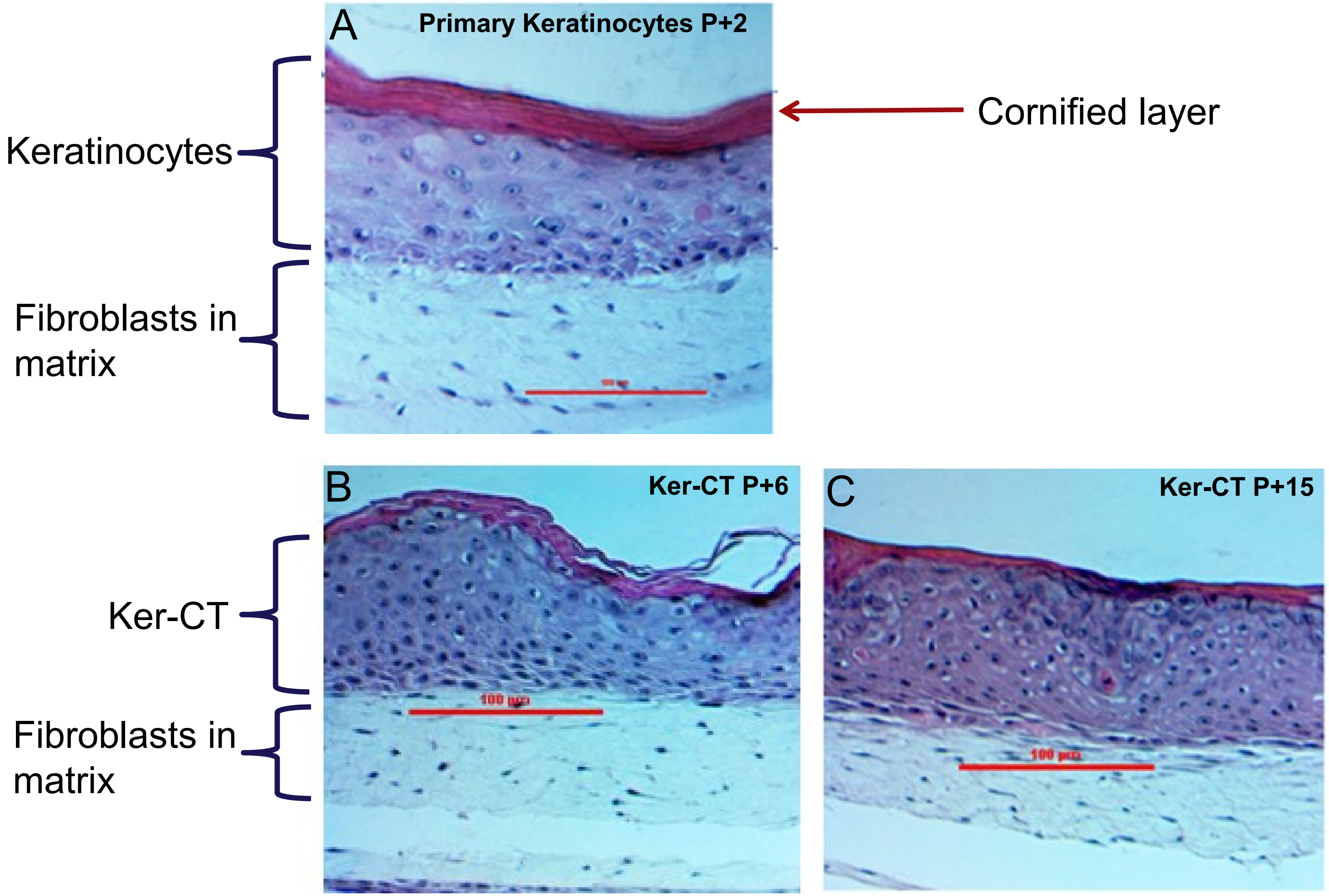
Late Differentiation
- Fillagrin

Early Differentiation
- Growth arrest and Keratin (KRT)

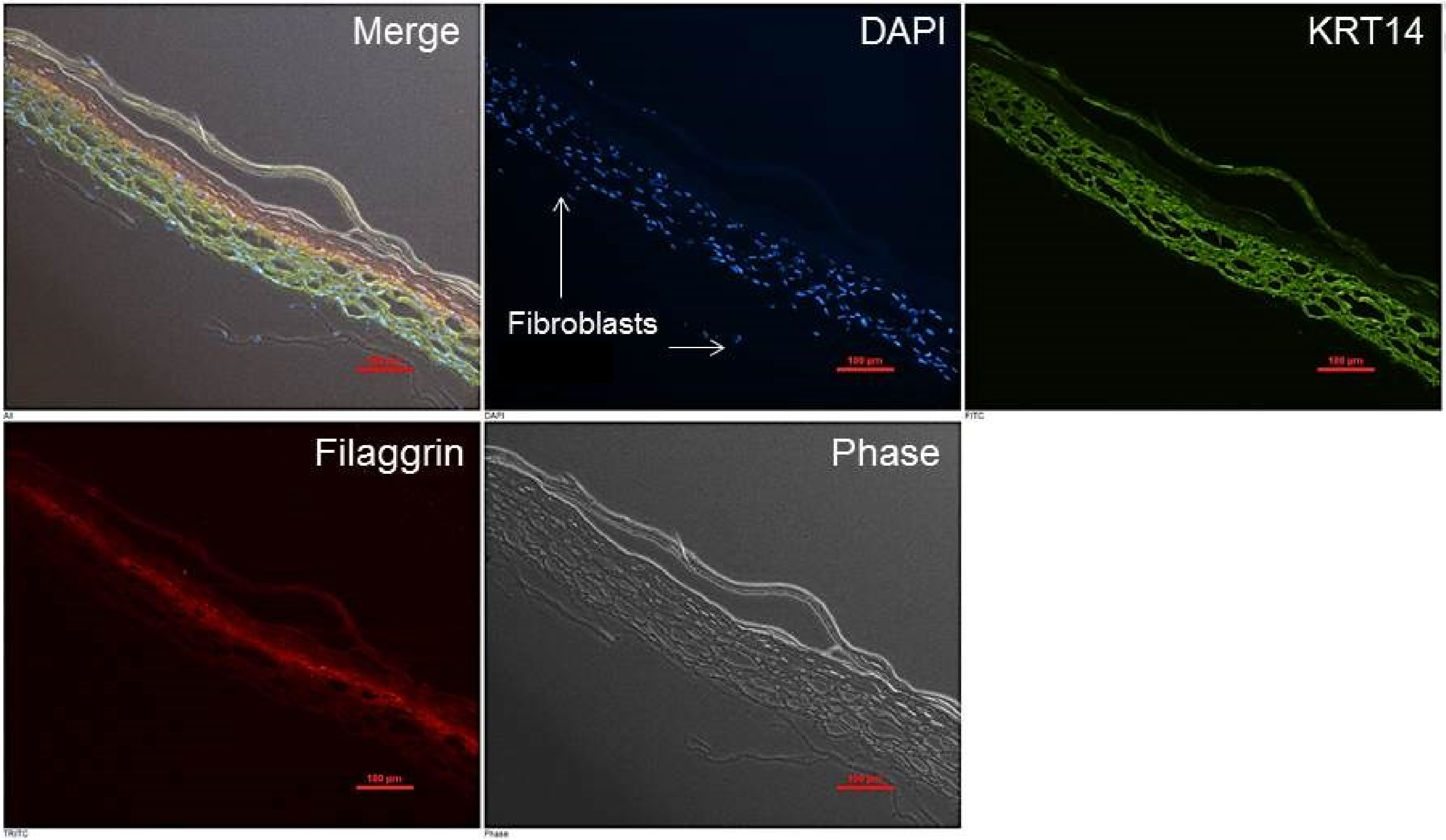
Proliferation
- DNA synthesis and mitosis
- KRT 5 and KRT 14

Adapted from Bollag B *et al*. Drug News and Perspectives 17(2):117-26, 2004.

Primary Keratinocyte and Ker-CT differentiation

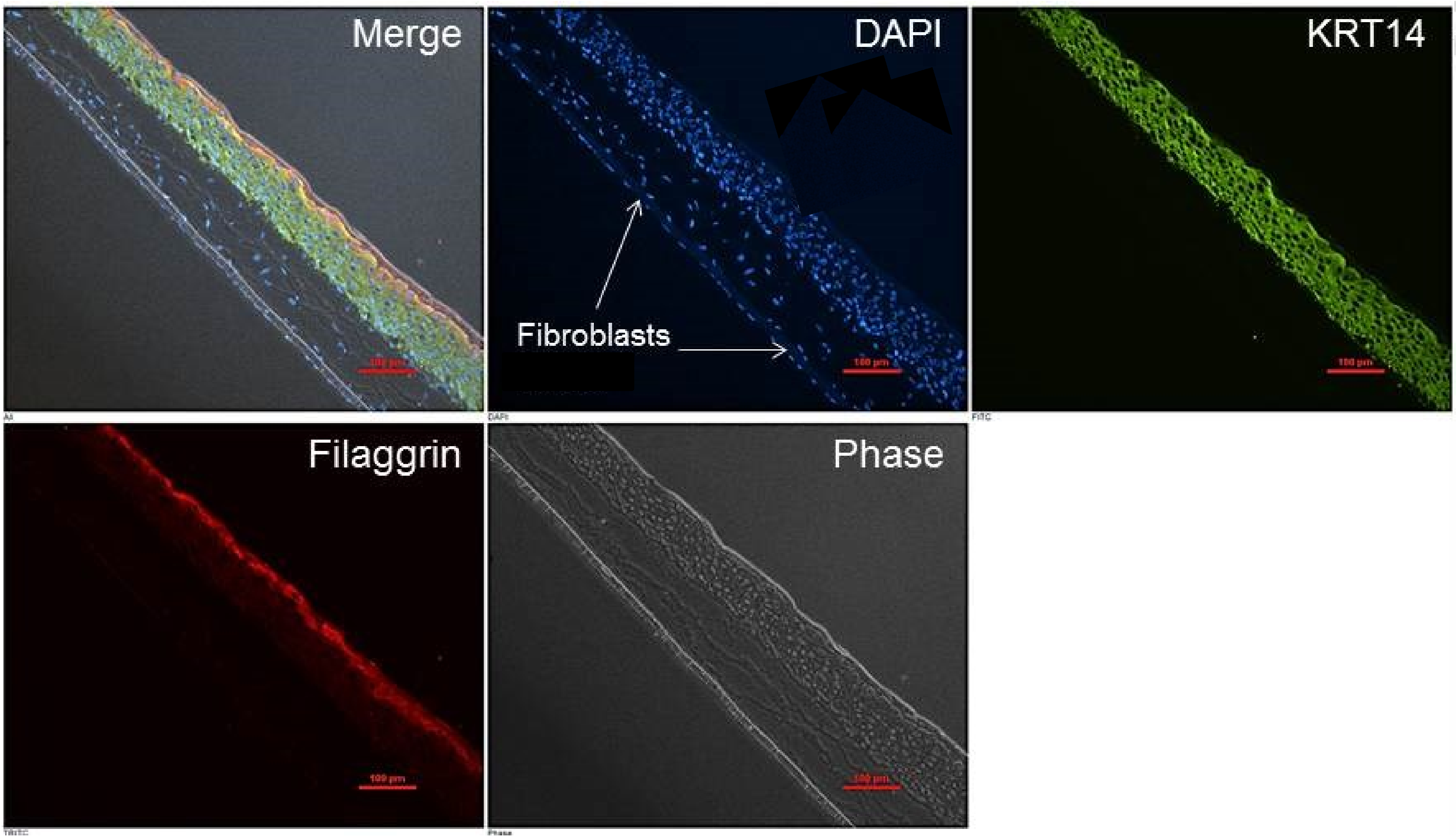


Immunohistochemistry of Primary Keratinocyte culture 11 days post airlift



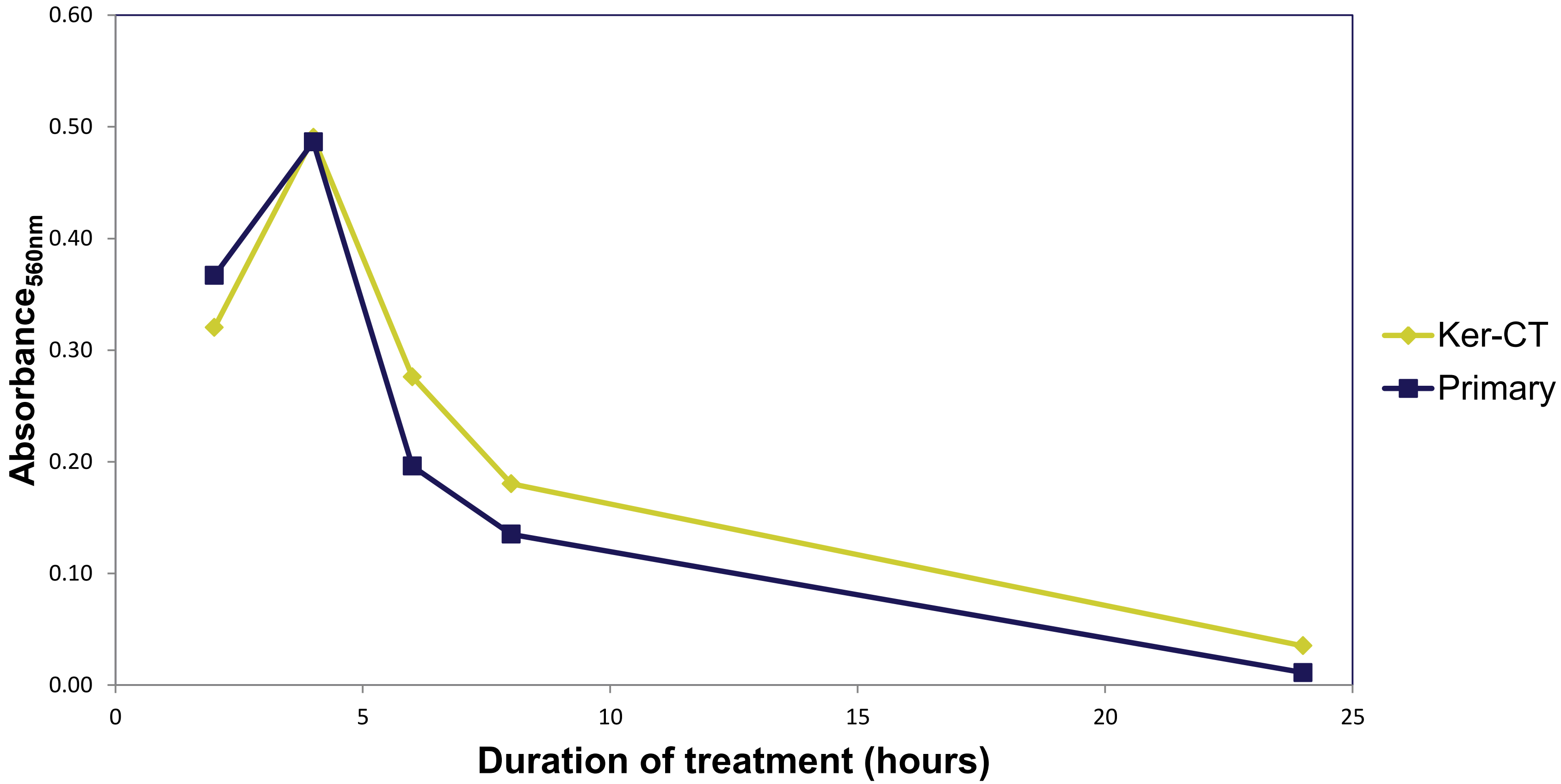
ATCC®

Immunohistochemistry of Ker-CT culture 11 days post airlift



ATCC®

Keratinocyte 3D skin model toxicology test with 1% Triton X-100™



Survival monitored by MTT Cell Proliferation Assay (ATCC® 30-1010K)

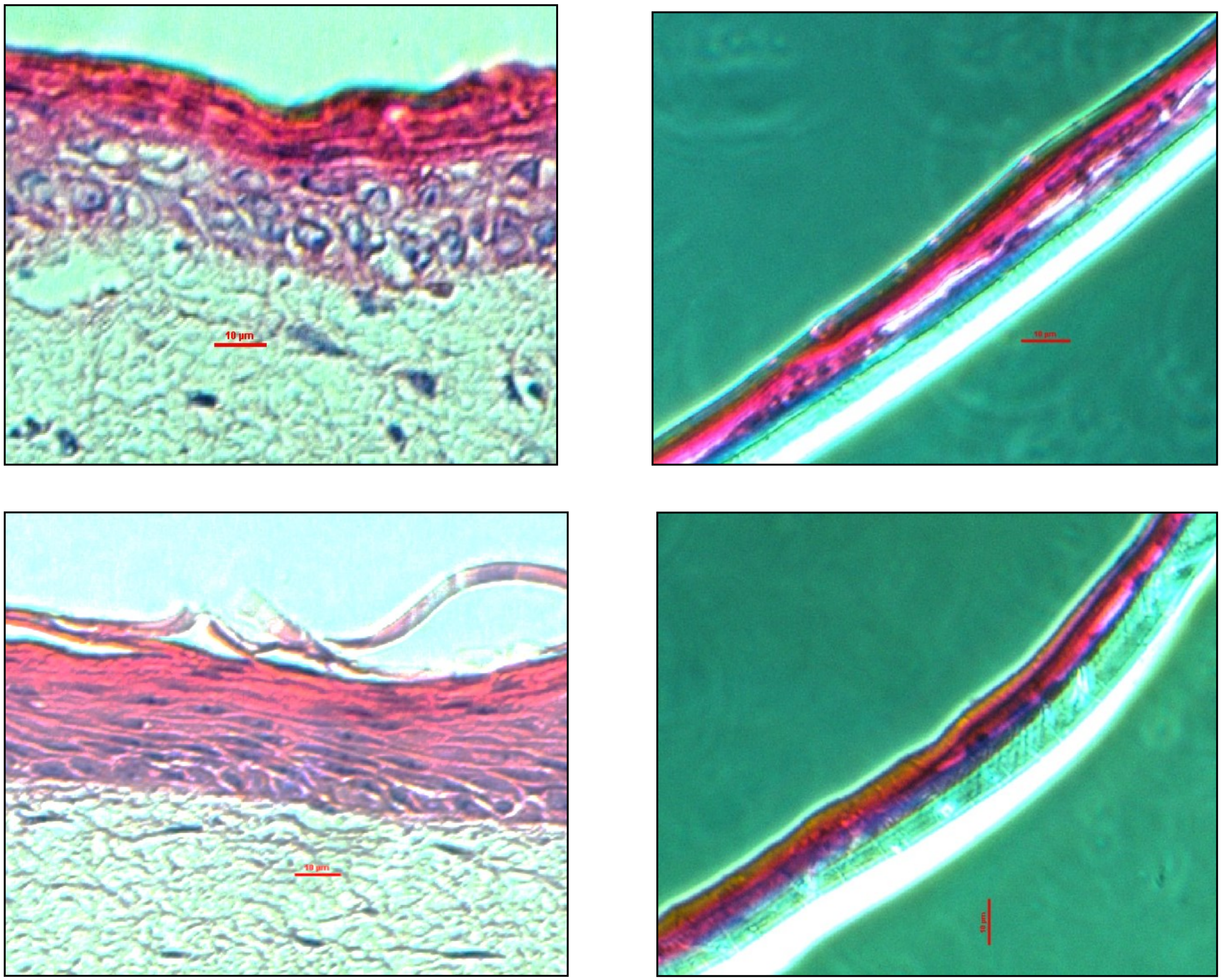
Keratinocytes 14 days post airlift

With raft

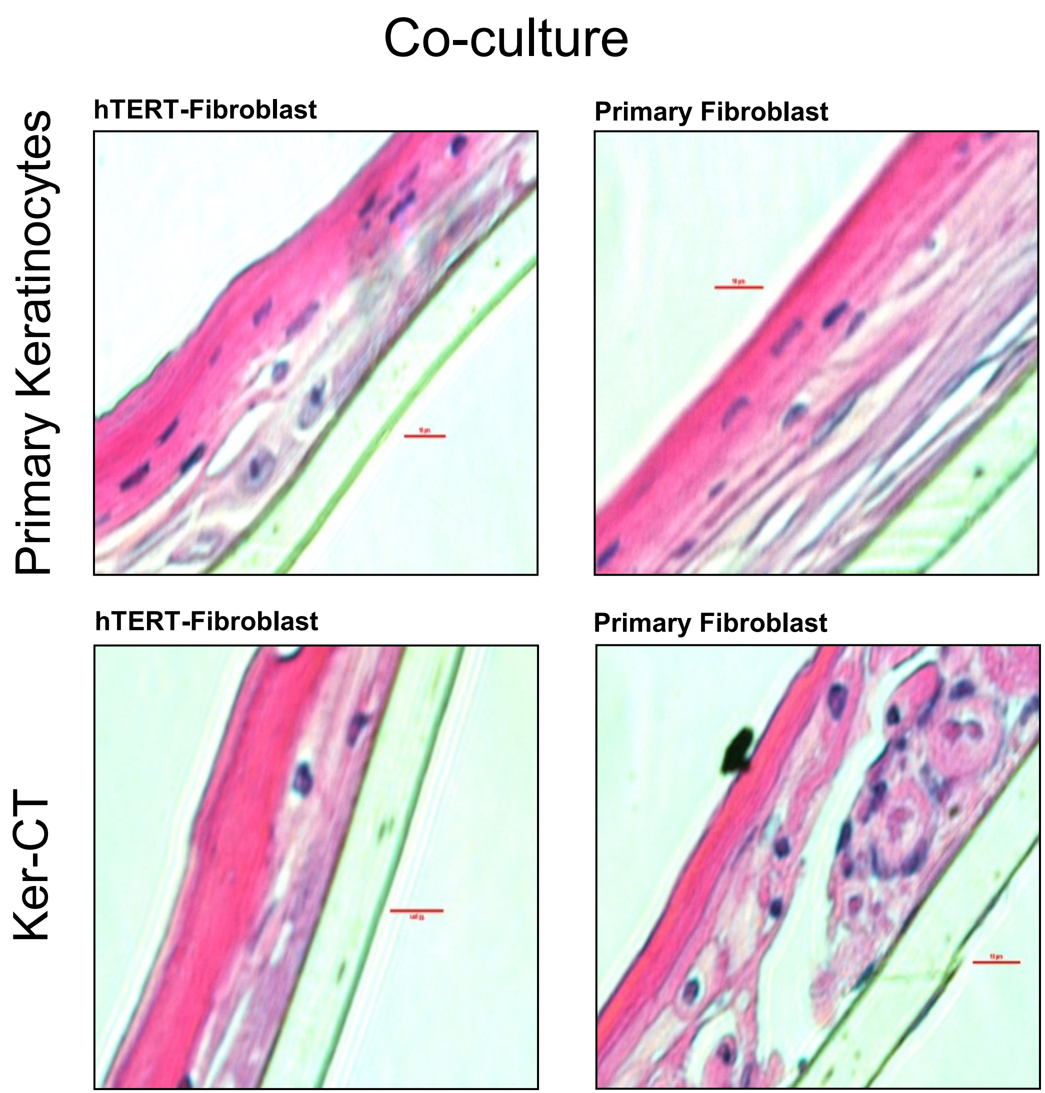
Without raft

Primary Keratinocytes

Ker-CT

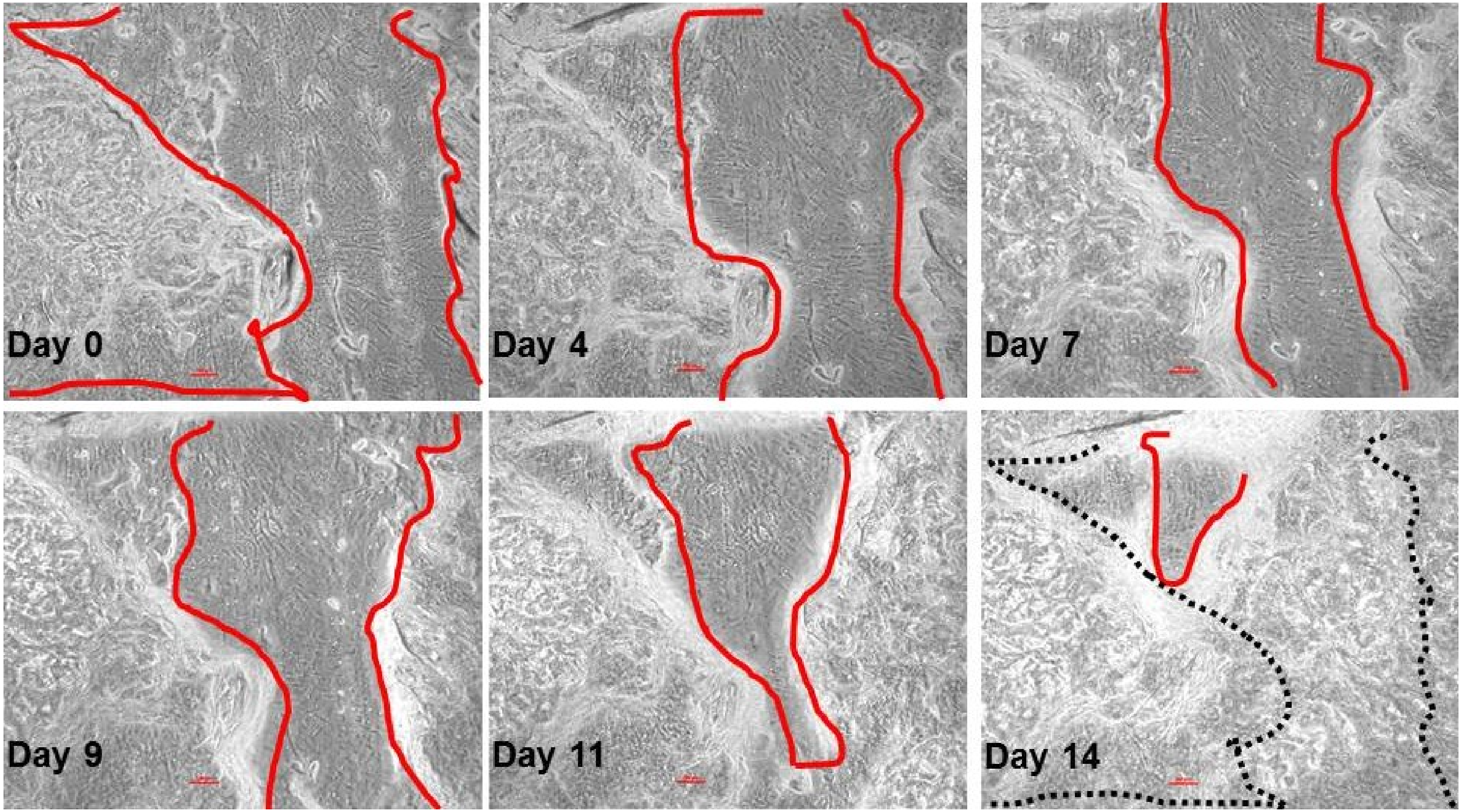


Primary Keratinocytes and Ker-CT 21 days post airlift



ATCC®

Scratch assay: Ker-CT co-culture with hTERT-MSCs, 21 days post airlift

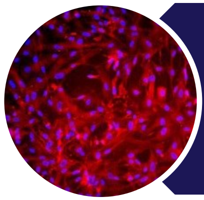


Summary: Dermatologic co-cultures

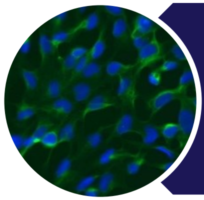
- Both primary and hTERT immortalized keratinocytes are viable resources for modeling skin
- Our raft co-culture supports growth and differentiation of primary and hTERT immortalized keratinocytes
- Keratinocyte co-cultures minus the raft are supported by fibroblasts
- Primary and immortalized co-culture models can be used to support skin toxicity studies – wound healing models may be supported by immortalized MSC co-cultures

ATCC®

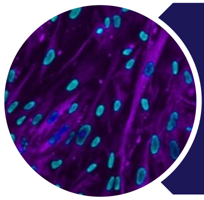
Outline



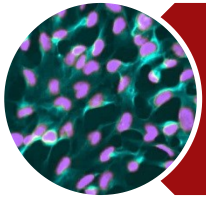
The significance of 3D culture



Air-liquid interface respiratory models



Dermatologic models



Angiogenesis models

ATCC®

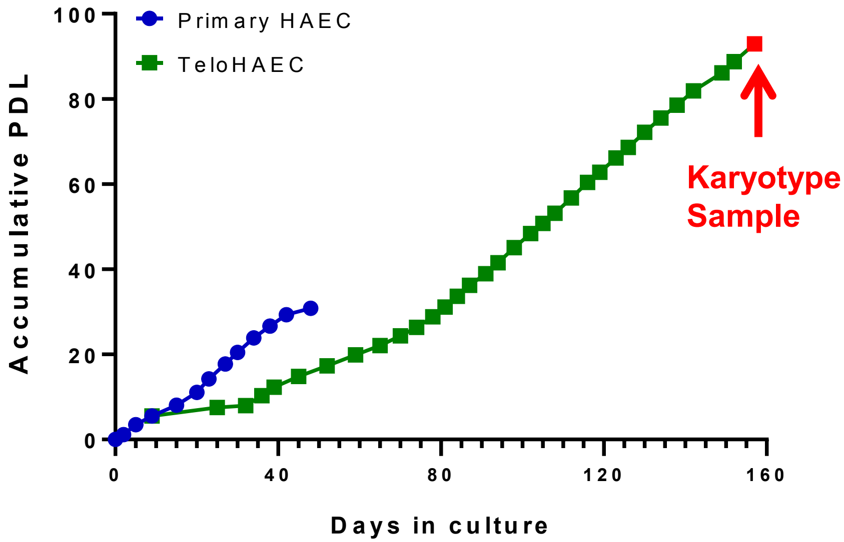
hTERT Immortalized Endothelial Cell Lines

- Express surface markers and receptors (PECAM-1/CD31, VEGFR2, Tie-2)
- Exhibit Ac-LDL uptake (LDL receptor functional assay)
- Demonstrate neoangiogenesis – Tubule formation on basement membrane gel

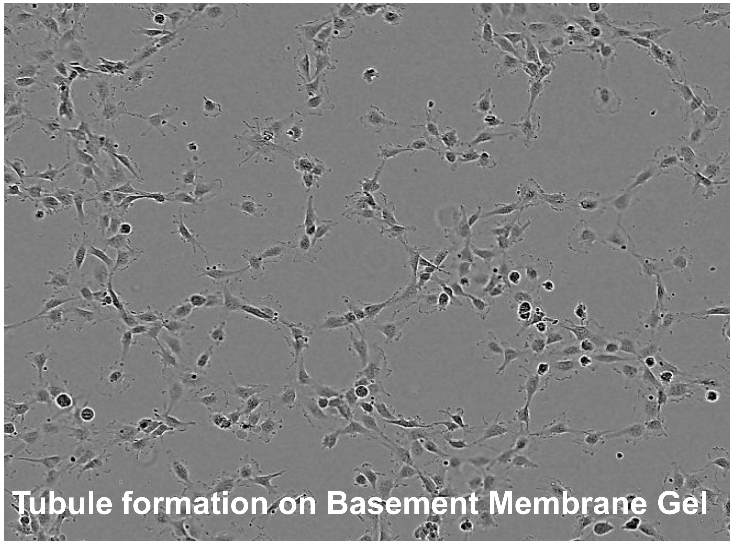
ATCC® No.	Cell Line	Description
CRL-4052™	**TeloHAEC**	Normal adult aortic endothelial cells
CRL-4025™	**TIME**	Foreskin microvascular endothelial cells
CRL-4045™	**TIME-GFP**	Foreskin microvascular endothelial cells with constitutive expression of EmGFP®
CRL-4049™	**NFkB-TIME**	Foreskin microvascular endothelial cells with NanoLuc® reporter expression under the control of NFkB response elements
CRL-4054™	**TeloHAEC-GFP**	Normal adult aortic endothelial cells with constitutive expression of EmGFP®

NanoLuc® is a trademark of Promega, and EmGFP® is a trademark of Life Technologies.

TeloHAEC – immortalized aortic endothelial cells



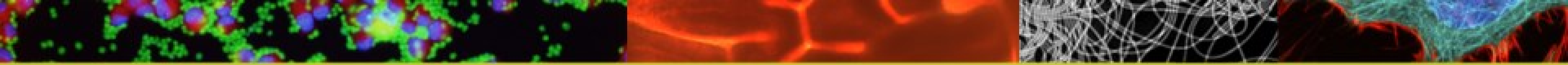
Normal Diploid Karyotype



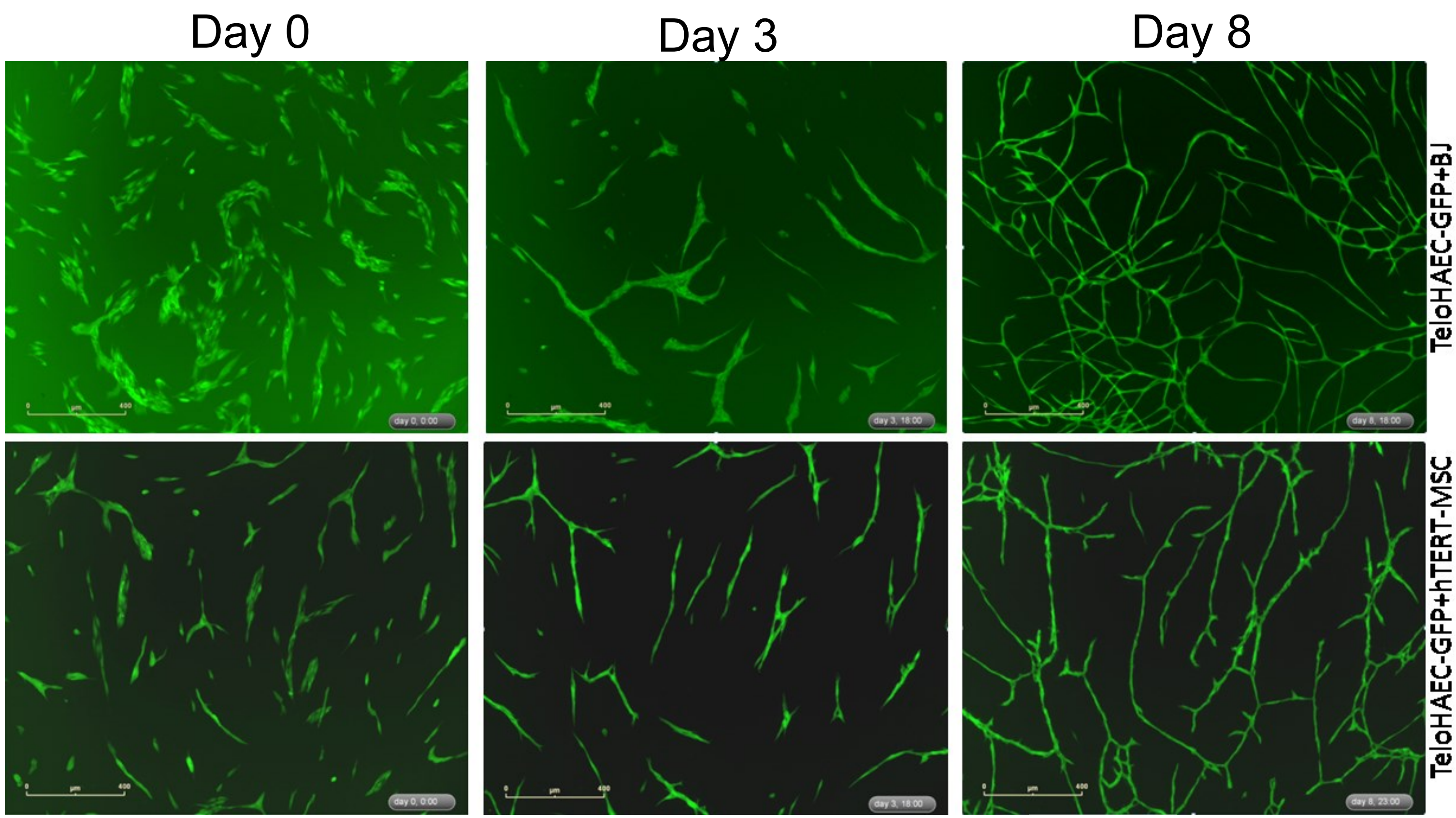
Tubule formation on Basement Membrane Gel

TeloHAEC	ATCC® CRL-4052™
Media	ATCC® PCS-100-030™
	ATCC® PCS-110-041™ (VEGF Kit)
Cell Basement Membrane Gel	ATCC® ACS-3035™

ATCC®

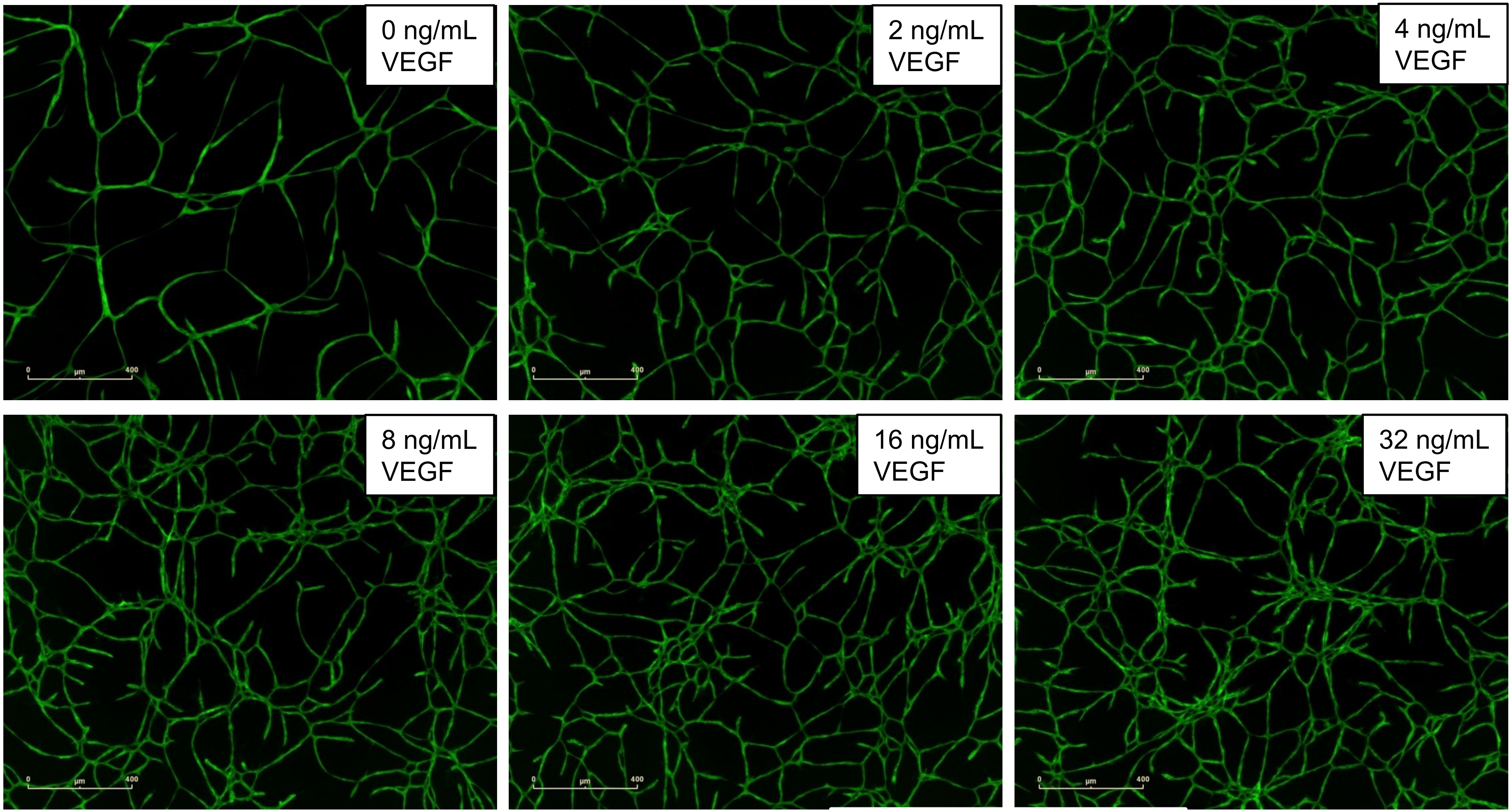


TeloHAEC-GFP co-cultured with BJ Fibroblast or hTERT-MSCs induces tubule formation



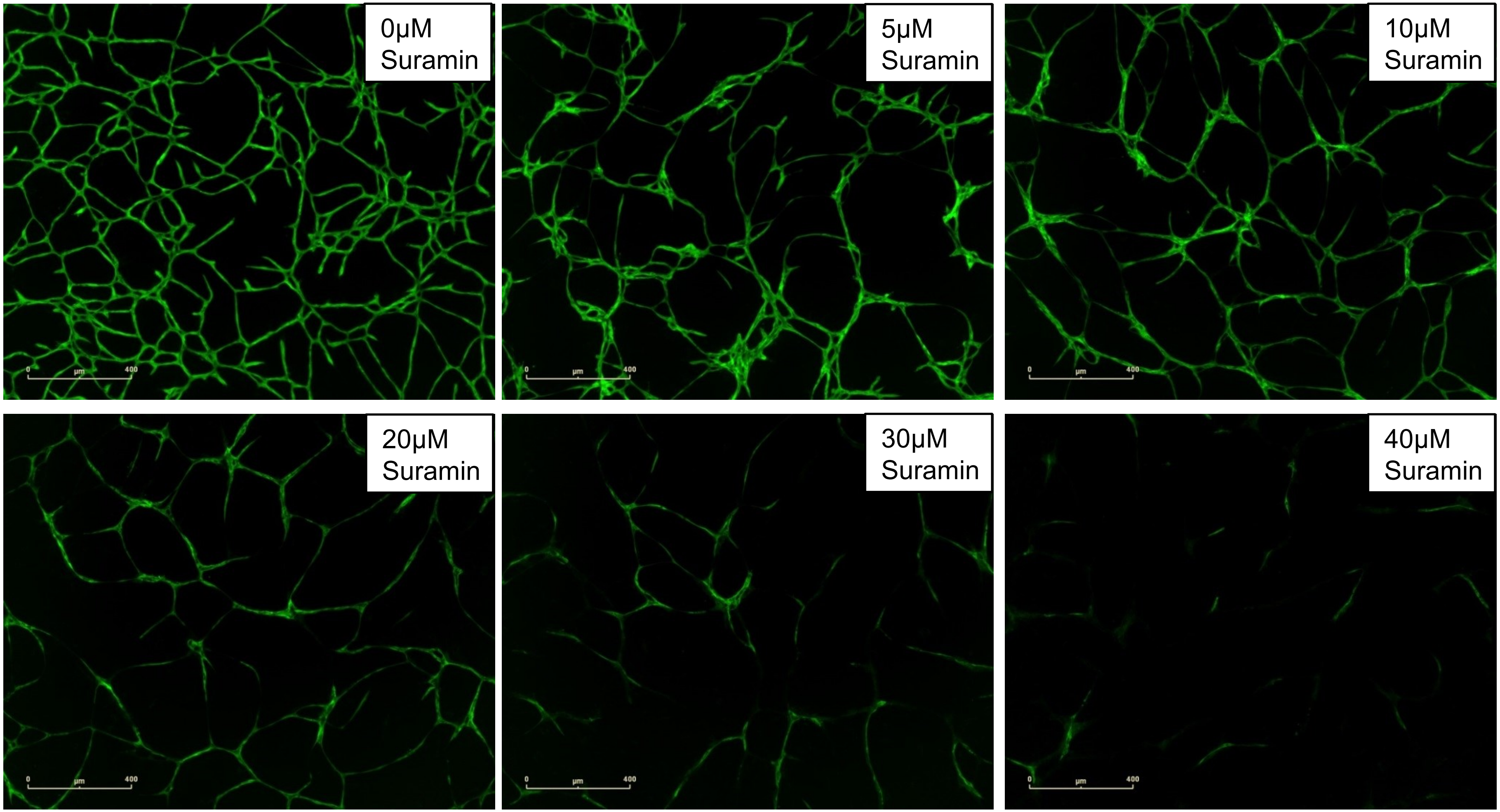
TeloHAEC-GFP (ATCC® CRL-4054™) co-cultured with BJ Fibroblasts (ATCC® CRL-2522™) or hTERT Adipose-derived MSC (ATCC® SCRC-4000™) in the ATCC® Angiogenesis Medium (coming soon) for 14 days.

VEGF stimulates tubule formation in the TeloHAEC-GFP and hTERT-MSC co-culture



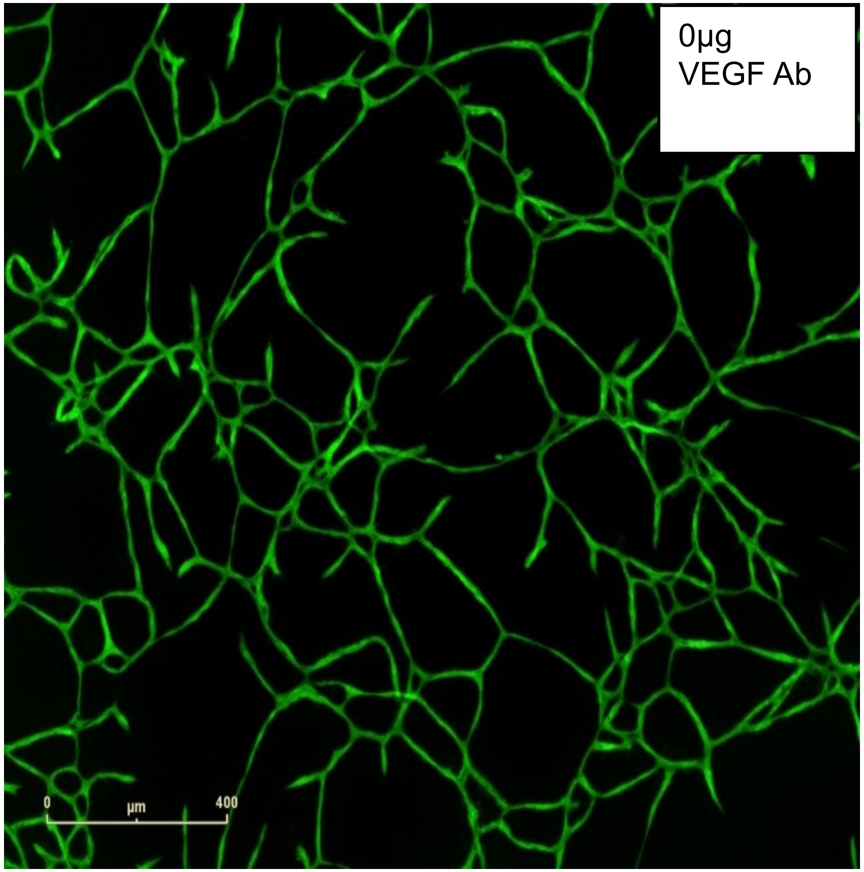
ATCC®

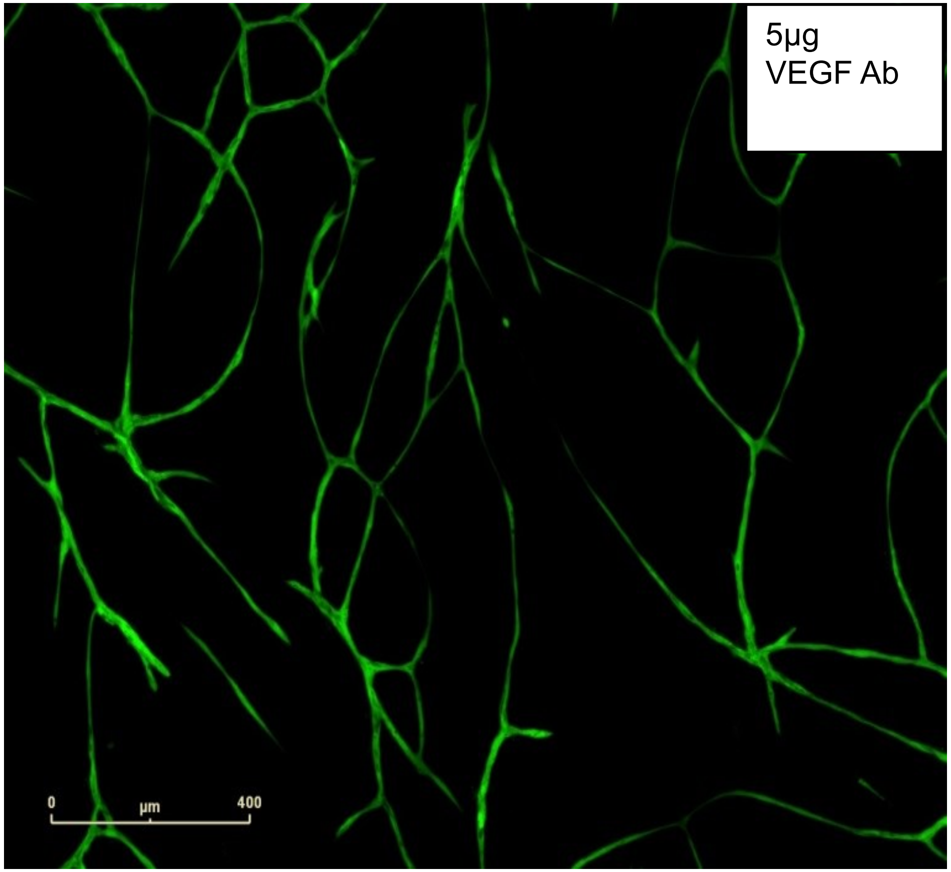
Suramin blocks tubular structure growth in TeloHAEC-GFP and hTERT-MSC co-culture



ATCC®

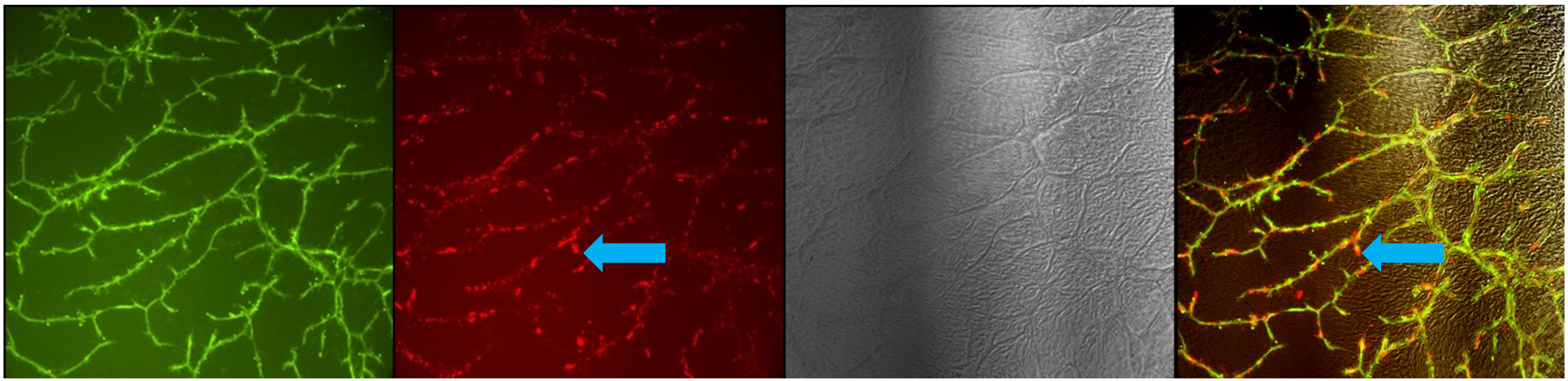
VEGF Ab blocks tubular structure growth in TeloHAEC-GFP and hTERT-MSC co-cultures





ATCC®

hTERT-MSC transformation to smooth muscle cells supports angiogenesis



GFP Anti-αSMA Phase Merged

- hTERT-MSC transformation to smooth muscle cells - indicated by α-SMA staining on the periphery of the TeloHAEC-GFP cells (arrows).
- Data may reflect similar conditions to angiogenesis occurring *in vivo.*

ATCC®

Conclusions

- 3D culture can provide a model system which reflects the phenotypic characteristic and genetic backgrounds of the *in vivo* tissue microenvironment.
- Both primary and hTERT immortalized cells can be used to support 3D modeling.
- ATCC is a resource for developing respiratory, dermatologic, and angiogenesis 3D co-culture models.

Thank you!

Watch recorded ATCC® "*Excellence in Research*" webinars on demand at www.atcc.org/webinars.

The ATCC® *"Excellence in Research"* webinar series returns in Spring 2015. Look for webinars starting in February at www.atcc.org/webinars.

Thank you for joining today!
Please send additional questions to tech@atcc.org

ATCC®