Universal extracellular matrix supports toxicology and drug screening in 2D and 3D cell culture models from iPSC-derived cells, primary cells, engineered lines, and cancer lines

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Abstract

In vitro cell culture models are becoming increasingly complex in an effort to better mimic *in vivo* physiology and enhance their predictive power and relevance in areas including absorption, distribution, metabolism, excretion, and toxicity assays (ADMET), drug discovery, phenotypic screening, developmental biology, and basic research. One major advance in model development has been the recognition of the critical role of the cell microenvironment including the presence of extracellular biomolecules, cell-to-cell interactions, and physical properties of the substrate. These elements can influence a variety of cellular functions including cellular metabolism, differentiation potential, gene/protein expression, drug susceptibility, proliferation, and survival, typically with extracellular matrix (ECM)- and cell type-specificity. Herein we report the development of a "universal" CellMatrix Basement Membrane ECM that provides a suitable microenvironment to support a wide variety of cell biology applications. Our results show that with optimized protocols the ECM permitted the long-term 3D culture of primary- and iPSC (induced pluripotent stem cells)- derived gastric and intestinal (GI) organoids from human and mouse cells and significantly enhanced spheroid forming efficiency in multiple continuous cancer lines. ATCC CellMatrix Basement Membrane also supported angiogenic vessel formation in engineered, immortalized and primary human endothelial cells under co-culture conditions; increased cytochrome P450 enzyme activity and multidrug resistance-associated protein 2 (MRP2) expression in "sandwich" culture of primary human hepatocytes; and allowed the routine culture of iPSCs and NPCs (neural progenitor cells) under serum- and feeder-free conditions while maintaining and promoting the capacity for terminal differentiation into neurons and beating cardiomyocytes. These results demonstrate that ATCC CellMatrix Basement Membrane provides a suitable biological matrix for advanced, physiologically relevant *in vitro* cell- based models and assays.

Methods

Extracellular matrix: ATCC CellMatrix Basement Membrane (CellMatrix) was stored at -80°C prior to use. Protein concentration ranged from 12 to 18 mg/mL. Please refer to the certificate of analysis for the lot-specific concentration. To thaw, stock vial was placed at 4°C overnight and aliquoted and stored at -80°C. Immediately prior to use, CellMatrix aliquots were thawed and kept on ice.

Description	ATCC© No.	Size
CellMatrix Basement Membrane; Growth factor reduced; LDEV-free	ACS-3035	5 mL

Hepatocyte culture: Cryopreserved primary human hepatocytes were thawed and plated on collagen coated 24-well plates in primary human hepatocyte media (Thermo Fisher Scientific). Six hours after plating the media was removed and an overlay of ATCC CellMatrix was added at a concentration of 250 µg/mL. Media was changed daily. Images were captured via phase contrast and immunofluorescent microscopy with a 10X objective.

iPSC and NPC culture: ATCC iPSCs were cultured according to the ATCC Stem Cell Culture Guide[1]. NPCs were handled and differentiated as previously described[2]. Both iPSCs and NPCs were routinely maintained on CellMatrix coated dishes and plates at a concentration of 150 µg/mL. ATCC iPSCs were differentiated to beating cardiomyocytes using published protocols[3]. Images were captured via phase contrast and immunofluorescent microscopy with a 10X or 20X objective.

Description	ATCC© No.	Size
Human Induced Pluripotent Stem Cells	Various	>1x10^6 cells
Neural Progenitor Cells; Normal, Human	ACS-5003	>1x10^6 cells
Neural Progenitor Cells; Normal, Human; MAP2-NanoLuc®- HaloTag®	ACS-5007	>1x10^6 cells
Neural Progenitor Cell Expansion Growth Kit	ACS-3003	500 mL
Neural Progenitor Cell Dopaminergic Differentiation Kit	ACS-3004	250 mL

Organoid culture: Human iPSC-derived organoids were generated and cultured as described elsewhere[4] and maintained in 100% CellMatrix in 3D. Primary murine small intestine-derived organoids were derived from LRG5 + stem cells cultured in 100% CellMatrix in 3D according to published protocols[5]. Images were captured via bright field and immunofluorescent microscopy or confocal microscopy with a 4X,10X or 20X objective.

Spheroid formation: Continuous cancer cell lines were thawed and cultured according to the respective ATCC product sheet. In ultra-low attachment round bottom plates, 1000 cells per well were seeded in complete growth medium containing 2.5% CellMatrix. Spheroids appeared within 24 hours and were monitored for 96 hours. Images were captured via phase contrast microscopy with a 10X objective.

Description	ATCC© No.
MCF7; Mammary epithelial adenocarcinoma	HTB-22
A549; Lung epithelial carcinoma	CCL-185
MDA-MB-231; Mammary epithelial adenocarcinoma	HTB-26
Hep G2; Hepatocellular carcinoma	HB-8065

Angiogenesis: Primary or immortalized endothelial cells, some constitutively expressing GFP, were seeded on CellMatrix coated (10 mg/mL) multiwell plates as previously described [6]. Tubules formed within 6 hours and could persist for 10+ days in culture. Images were captured via fluorescent microscopy with a 4X objective.

Description	ATCC© No.	Size
Primary Umbilical Vein Endothelial Cells; Normal, Human	PCS-100-010	>0.5x10^6 cells
Primary Aortic Endothelial Cells; Normal, Human	PCS-100-011	>0.5x10^6 cells
TeloHAEC-GFP; immortalized human airway epithelial cells expressing GFP	CRL-4054	>1x10^6 cells
TIME-GFP; immortalized human dermal microvascular endothelial cells expressing GFP	CRL-4045	>1x10^6 cells

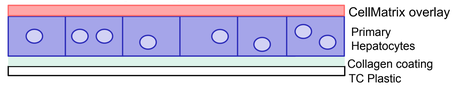
Results

ATCC CellMatrix Basement Membrane is purified from the murine Engelbreth Holm Swarm sarcoma. Major components include laminin, collagen IV, entactin, and heparin sulfate proteoglycan. CellMatrix was found to support or enhance a broad range of *in vitro* 2D and 3D cell culture models for use in drug discovery, toxicology, and basic research by providing a more *in vivo*-like microenvironment for improved physiological functionality, response, and relevance.

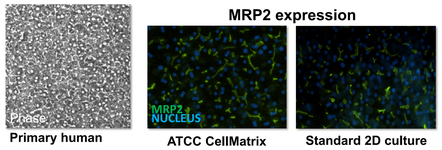
Hepatocytes

Sandwich culture of primary human hepatocytes using CellMatrix overlay provides an *in vivo*-like microenvironment that results in enhanced *in vitro* functionality.

Schematic depicting sandwich culture conditions

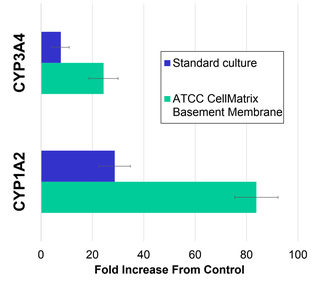


MRP2 expression



Primary human hepatocytes | ATCC CellMatrix sandwich culture | Standard 2D culture

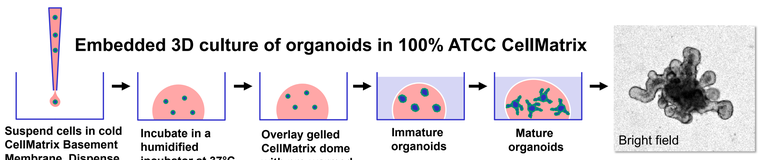
Cytochrome P450 Enzyme Induction

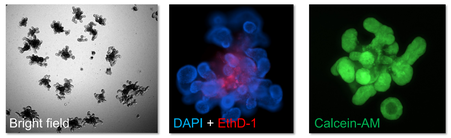


Organoids

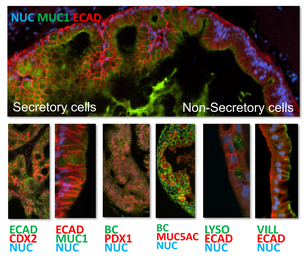
GI organoids from primary tissue and multiple human ATCC iPSCs exhibited gastric- and intestine-like features after culture in serum free, defined media and embedded in CellMatrix.

Embedded 3D culture of organoids in 100% ATCC CellMatrix



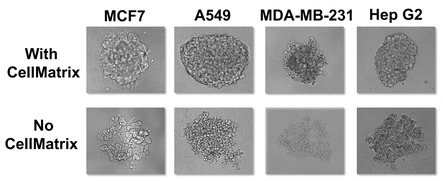


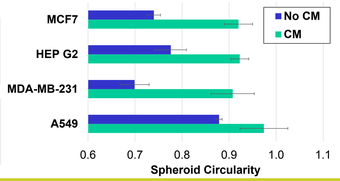
Organoids embedded in 100% ATCC CellMatrix (above left) can be stained with live/dead cell dyes (DAPI or Calcein-AM for viable cells and EthD-1 for dead cells) and imaged by confocal microscopy (above middle and above right) or fixed, sectioned and stained with immunofluorescent antibodies (right). Mature GI organoids expressed many typical tissue GI markers for cell types including enterocytes, goblet cells, and Paneth cells.



Spheroids

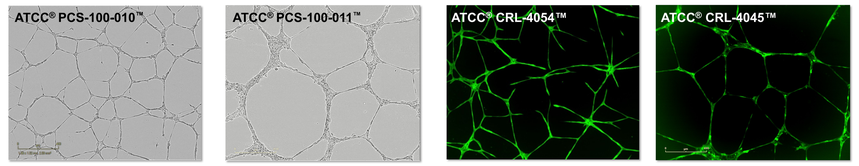
Spheroid formation is enhanced by the addition of CellMatrix in multiple cancer cell lines. Spheroids formed with CellMatrix are more spherical (below) and develop faster (data not shown) than when cultured alone.





Angiogenesis

Primary, immortalized, and engineered endothelial cells constitutively expressing GFP form networks of tubules when seeded on CellMatrix Basement Membrane. Angiogenesis can be stimulated or inhibited with known regulators such as VEGF and suramin (data not shown) and monitored in real-time.

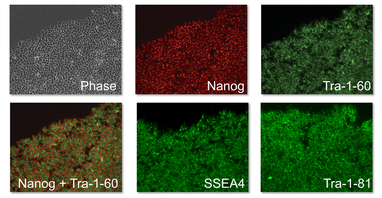


Primary HUVEC | Primary Aortic | TeloHAEC-GFP | TIME-GFP

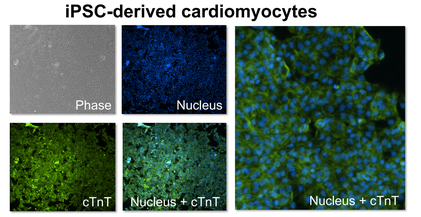
iPSC-derived NPCS, neurons, astrocytes, and cardiomyocytes

ATCC CellMatrix provided a supportive microenvironment for the xeno- and serum-free culture of iPSCs as well as iPSC-derived cell types including NPCs, neurons, and spontaneously beating cardiomyocytes. Differentiated cells (neurons vs. NPCs) show differential sensitivity to various compounds.

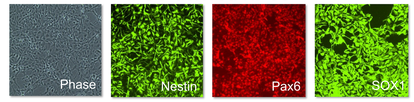
iPSCs



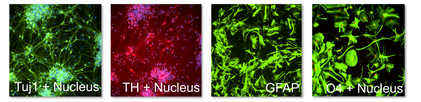
iPSC-derived cardiomyocytes



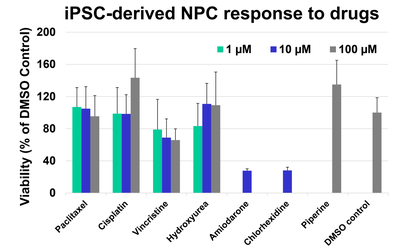
iPSC-derived NPCs



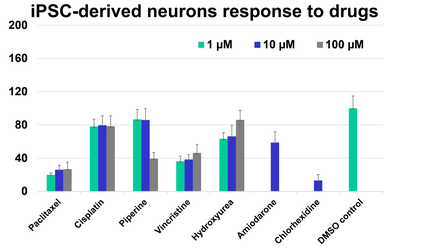
iPSC-derived neurons, astrocytes, and oligodendrocytes



iPSC-derived NPC response to drugs



iPSC-derived neurons response to drugs



Summary

ATCC CellMatrix Basement Membrane Gel supports numerous cell biology applications that are relevant for drug screening, toxicology, and basic research including hepatocyte culture, organoid generation, cancer research, 3D culture of cancer cells, and human stem cell maintenance and differentiation into neural progenitor cells, neurons, astrocytes, oligodendrocytes, and cardiomyocytes. Visit the ATCC website and our ASCB booth for additional details on the various CellMatrix applications described here.

References

1. ATCC® Stem Cell Culture Guide: https://www.atcc.org/Guides/Stem_Cell_Culture_Guide.aspx
2. ATCC Application Note 23, Comprehensive gene expression analysis and neurotoxicity testing of human iPSC-derived neural progenitor cells and neurons. http://www.atcc.org
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5. Sato T, *et al.* Single Lgr5 stem cells build crypt villus structures *in vitro* without a mesenchymal niche. *Nature* 459(7244): 262-265, 2009. PubMed: 19329995
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