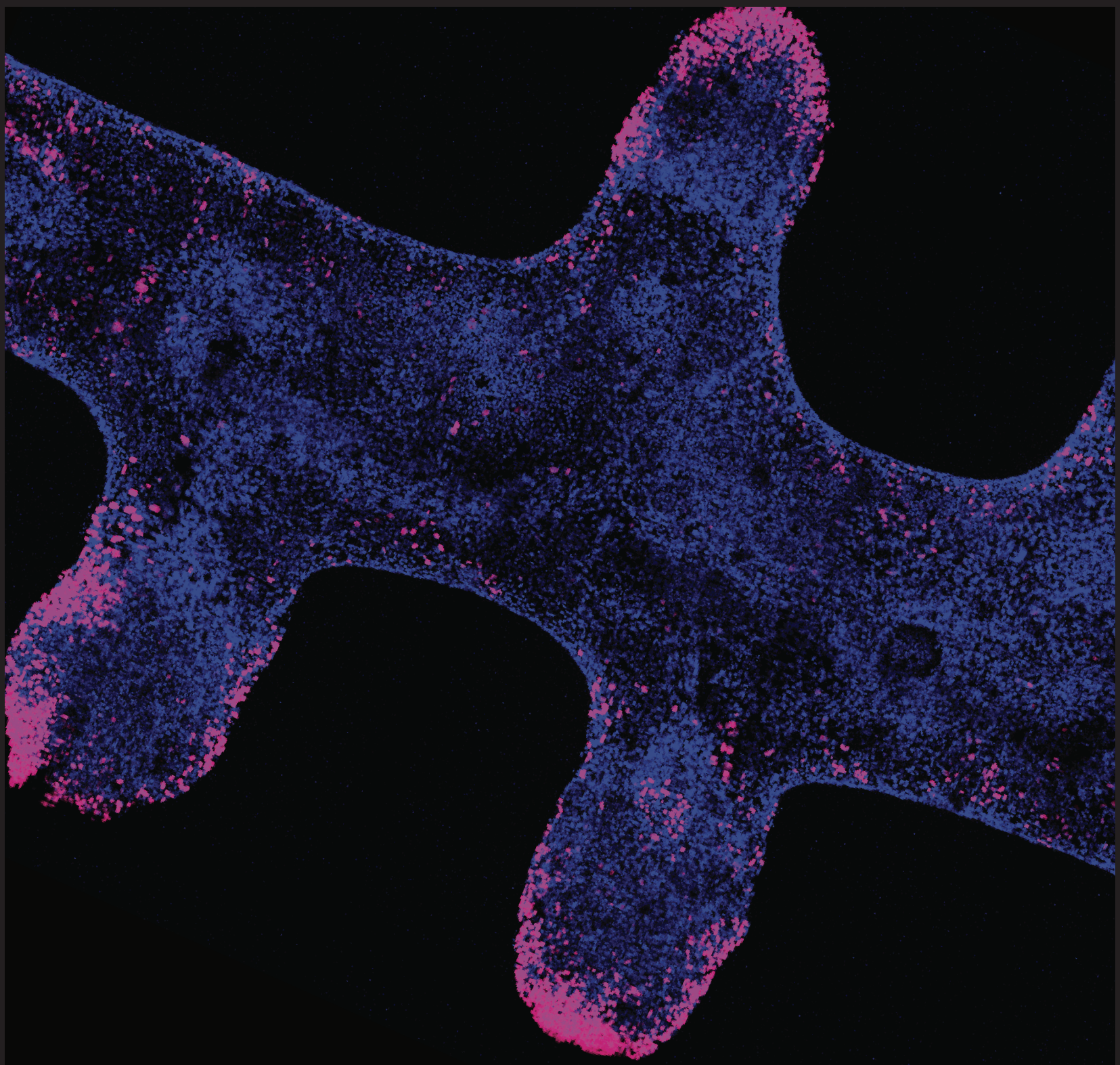

Organo Biotech

Simple Platform for Complex Biology

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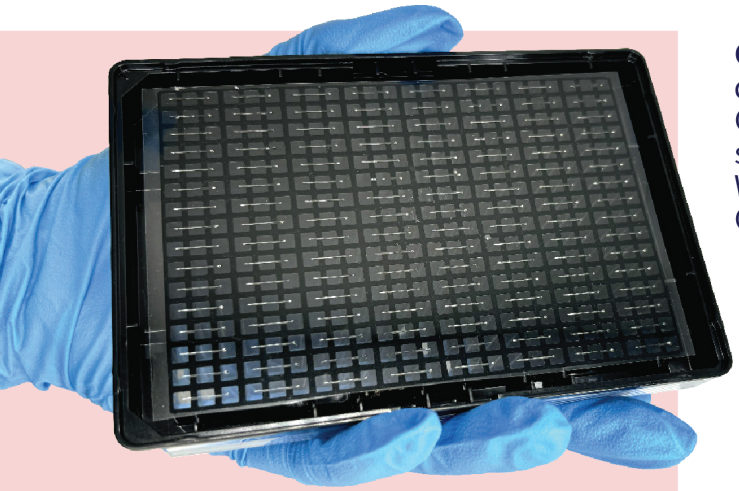
Simple Platform for Complex Biology



OrganoPlatform

OrganoPlatforms

- IFlowPlate384
- AngioPlate384
- UniPlate384
- UniPlate384 II

Supporting Equipments

- IFlowRocker
- AngioTEER
- AeroLung

OrganoAssays

- Blood brain barrier and vascular disease
- Colon model for inflammatory bowel disease
- Airway model for Asthma and viral infection
- Alveoli model for pulmonary fibrosis
- Kidney model for renal fibrosis and toxicity screening
- Placenta model for maternal-to-fetus drug transport
- Cancer model for immune-oncology.

ORGANOBIOTECH, Inc. aims to transform the way we develop drugs by combining automated high-throughput Organ-on-a-Chip technology, which mimics human organ systems, with the analytical power of machine learning. We generate values across three levels: OrganoPlatform, OrganoAssay, and OrganoDrug.

- We provide tissue culture consumables (OrganoPlatform) and supporting equipment for users to develop diverse 3D tissue models and assays on a high-throughput platform
- We provide research contract services on a wide range of functional assays (OrganoAssay) to recapitulate diseases in different types of tissues
- We identify promising drug candidates (OrganoDrug) by applying machine learning to extract insights from high-throughput Organ-on-a-Chip experiments

NEED: Despite pharmaceutical companies spending over $150 billion annually on drug discovery, the success rate in clinical trials is only about 9.6%. This low efficiency makes drug development expensive. As such, even a small improvement in predicting successful drug candidates at the pre-clinical stage could result in multi-billion dollar savings. Nonetheless, traditional methods relying on animal models and 2D cell cultures often fail to replicate human physiological response accurately.

SOLUTION: To improve drug discovery, we are adopting 3D human tissue models that more closely mimic human biology. Organ-on-a-Chip technology allows for the creation of tissue-specific structures and simulates the dynamic conditions of real organs by applying mechanical forces and fluid flows. This provides a realistic environment for testing drugs.

COMPETITIVE ADVANTAGE: Traditional organ-on-a-chip systems, which are based on microfluidics, confine cells and tissues within microchannels and synthetic membranes. This is in stark contrast to the soft and free-form environment of natural tissues. We are one of the first to integrate 3D printing of sacrfitial materials in the familiar multi-well plate format (***OrganoPlatform***), which enables the cultivation of 3D tissues with organ-specific structures within natural hydrogels without the restrictions imposed by microfluidic channels or synthetic scaffolds/membranes. This approach enabled us and end-users to build better models on an automated high-throughput platform.

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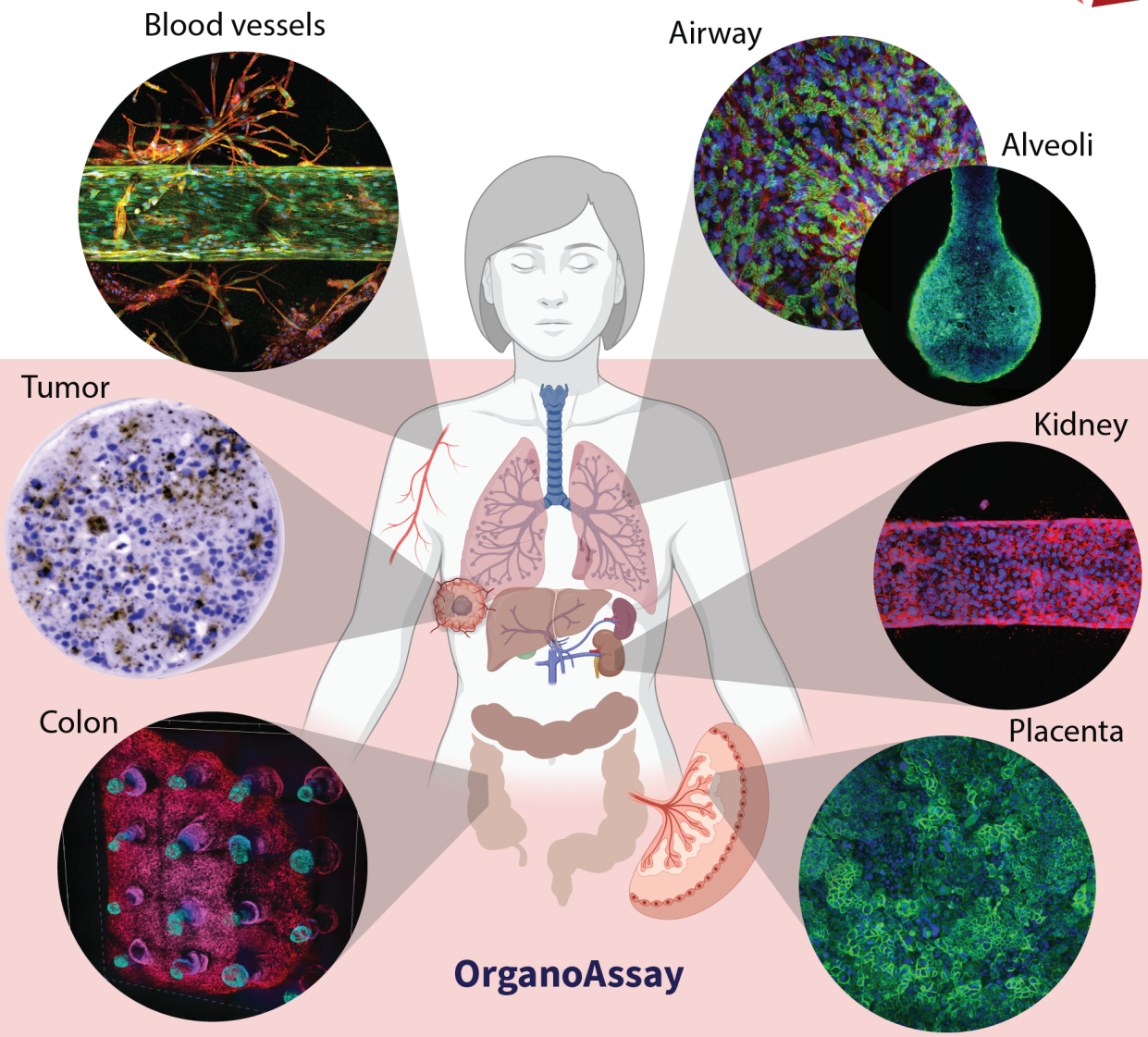
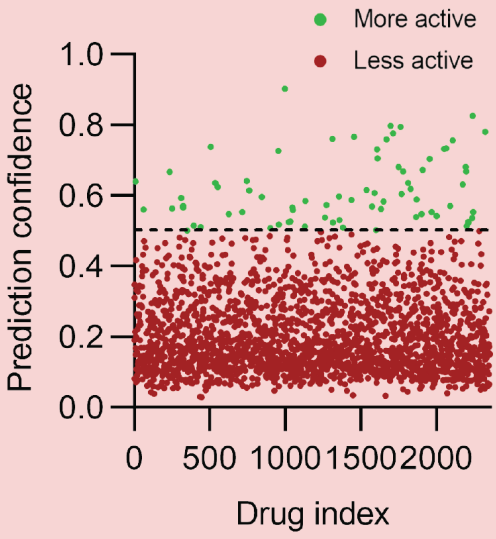


OrganoDrug: Leveraging the OrganoPlatform, we have developed a wide range of functional OrganoAssays to recapitulate diseases. But our ambition extends beyond assay development. We are discovering our own drug candidates utilizing the OrganoPlatform by honing our initial focus on pulmonary fibrosis, a condition that has surged in significance, especially following the COVID-19 pandemic. By integrating machine learning into our discovery pipeline, we've virtually screened 2353 comoounds, identifying promising candidates. These drug candidates are currently undergoing validation on our OrganoPlatform. Traditionally, this process from discovery to validation takes extensive time and substantial investment. We are achieving these milestones at accelerated pace and a reduced cost.



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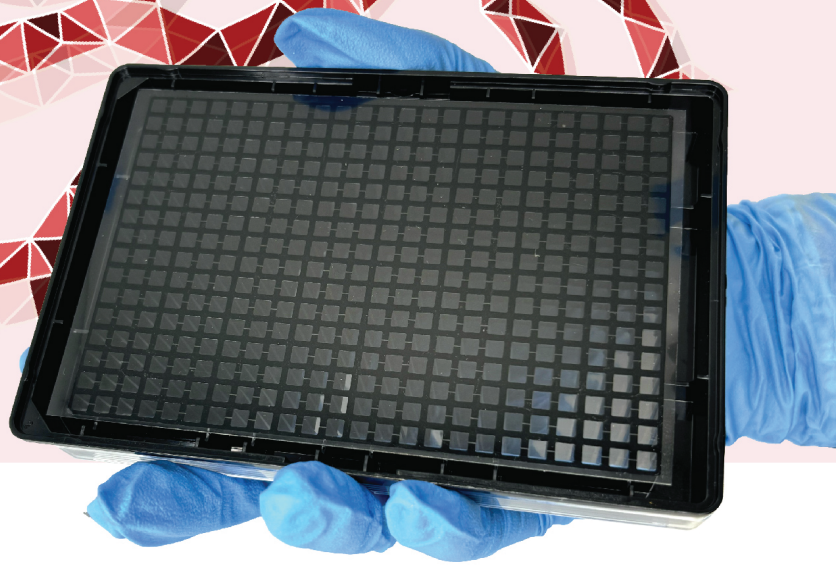
IFlowPlate384™



IFlowPlate is an organ-on-a-chip platform designed to replicate the complex microenvironment of human tissues with interstitial flow. Each IFlowPlate consists of 128 independent tissue culture units, offering the highest throughput available in the market and compatibility with automation workflows. Each unit includes an inlet, middle, and outlet well, interconnected by two channels. The simplistic design makes the platform highly versatile.

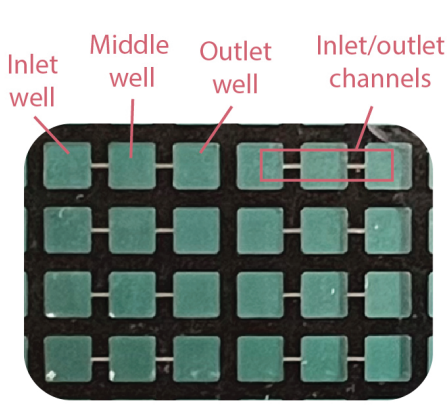
To create an epithelial tissue barrier model, a hydrogel can be cast in the middle well, with epithelial cells seeded on the gel surface and supporting cells embedded within. For controlling epithelial topography, ***TopoStamp*** (B003) introduces specific patterns on the gel surface. For vascularized organoids, endothelial cells and organoids can be embedded in the gel. For simple perfusion cultures of free-floating organoids or tissue spheroids, the organoids can be directly seeded into the middle wells without a gel.

The dimensions of the inlet and outlet channels are adjustable for specific applications. Narrower channels (A001) are ideal for physically connecting to self-assembled vasculature or trapping organoids and spheroids in the middle wells in the absence of a gel. Wider channels (A001-2) are recommended for ***AngioTEER*** measurements of tissue barriers. Bi-directional interstitial flow in the gel matrix is achieved with the IFlowRocker, removing the need for pumps.

The open-well design allows easy access for pipetting and tissue removal, facilitating downstream analysis. The optically clear plate bottom supports high-content imaging with confocal microscopy and high-throughput analysis with plate readers. The 384-well design minimizes the use of cells and reagents, significantly reducing experimental costs per data point. To learn more, visit www.organo-biotech.com/posters.

KEY FEATURES

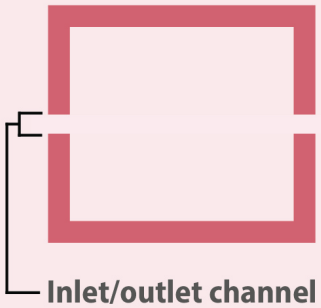
- 128 tissue culture units
- Pump-free bidirectional perfusion
- Membrane-free 3D tissue culture in hydrogel matrix
- Customizable inlet/outlet channel dimensions
- Automation compatible
- Low cell and media consumption



Bottom view of the plate

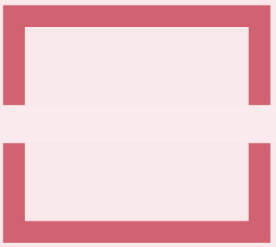
Customizable inlet/outlet channel sizes

A001



Inlet/outlet channel
0.2mm (W)
0.2mm (H)

A001-2



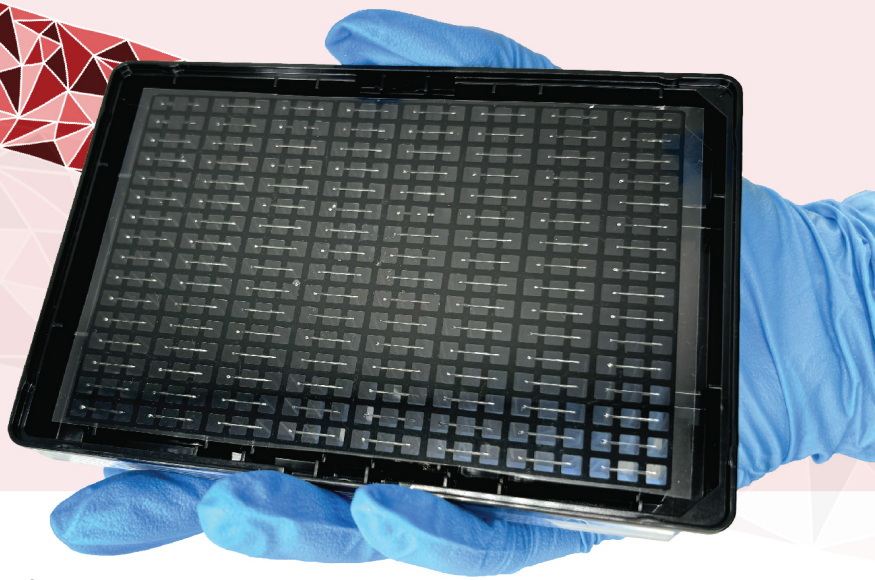
Inlet/outlet channel
0.5mm (W)
0.4mm (H)

Specifications

Product code	A001; A001-2
Number of culture per plate	128
Sterilization method	UV sterilized
Storage condition	Room temperature (15-25 °C)
Storage time	6 month
Plate format	SBS standard 384 well plate
Materials	Top plate: virgin polystyrene. Bottom plate: optical quality, low compound-absorbing plastic
Perfusion	Gravity driven perfusion with IFlowRocker (B001)
Applications	Perfused 3D tissue culture
Readouts	Sensor (TEER with AngioTEER, B002, suitable only with A001-2); Imaging (phase contrast, widefield fluorescence, confocal) ; plate reader (absorption, fluorescence, luminescence) ; off plate (Histology, ELISA, RNA/DNA analysis, MS, biochemistry)

Organo Biotech

AngioPlate384™



AngioPlate is an organ-on-a-chip platform designed to replicate the complex structure and function of human tissues. Each AngioPlate contains 128 independent tissue culture units, making it the highest-throughput system available in the market, and is compatible with automation workflows. Each unit contains a inlet, middle, and outlet well connected together *via* two channels. The middle well contains a 3D-printed sacrificial template that can be encapsulated by a natural hydrogel. Upon dissolving the templates, the system produces a perfusable structure within the hydrogel matrix, which can then be populated with cells. The absence of membranes between the perfusable structure and surrounding matrix enables direct interactions between cells to mimick physiological tissue environments.

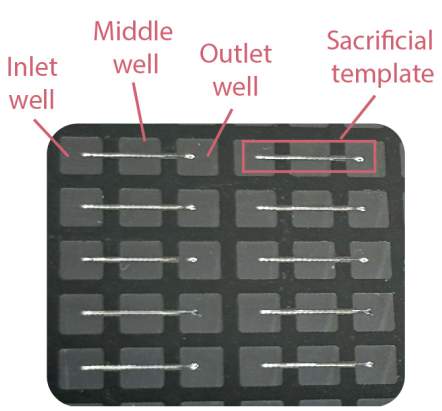
This versatile platform supports the creation of various tissue models, including tubular blood vessels, bifurcating vascular networks, renal epithelial tubules, colon tubes, and vascularized spheroids or organoids. These models can be used to study tissue barrier disruption, immune response, fibrosis and more in a highly controlled environment.

With customizable designs, including three standard templates and the option for tailored configurations, AngioPlate offers unparalleled flexibility for diverse research needs.

Bi-directional perfusion of tissues is achieved using the ***IFlowRocker***, eliminating the need for pumps. For unidirectional perfusion, researchers can explore the ***UniPlate384*** system. AngioPlate is compatible with the ***AngioTEER*** sensor, enabling real-time, continuous monitoring of tissue barrier integrity across 128 tissue units in a plate. The open-well design ensures easy access for pipetting and tissue removal for downstream analysis, while the optically clear plate bottom supports high-content imaging using confocal microscopy and high-throughput analysis with plate readers. The 384-well design also minimizes the use of cells and reagents to significantly reduce experimental costs per data point. To learn more, please visit *www.organo-biotech.com/posters.*

KEY FEATURES

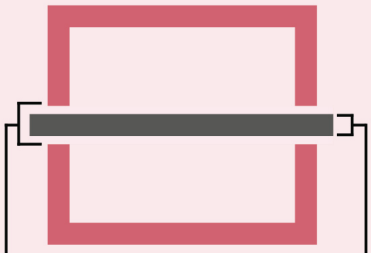
- 128 tissue culture units
- Pump-free bidirectional perfusion
- Membrane-free 3D tissue culture in hydrogel matrix
- Customizable tissue architecture
- Automation compatible
- Low cell and media consumption



Bottom view of the plate

Customizable tissue architecture

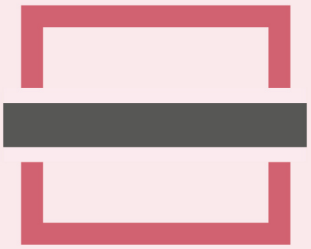
A002



Sacrifitial template
0.15-0.27mm (W)
0.05-0.1 mm (H)

Inlet/outlet channel
0.5mm (W)
0.4mm (H)

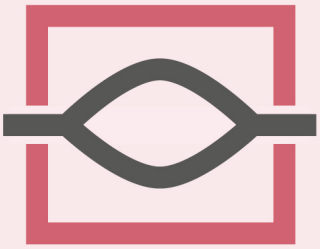
A002-2



Sacrifitial template
0.4-0.5mm (W)
0.2-0.3 mm (H)

Inlet/outlet channel
0.8mm (W)
0.5mm (H)

A002-3



Sacrifitial template
0.15-0.27mm (W)
0.05-0.1 mm (H)

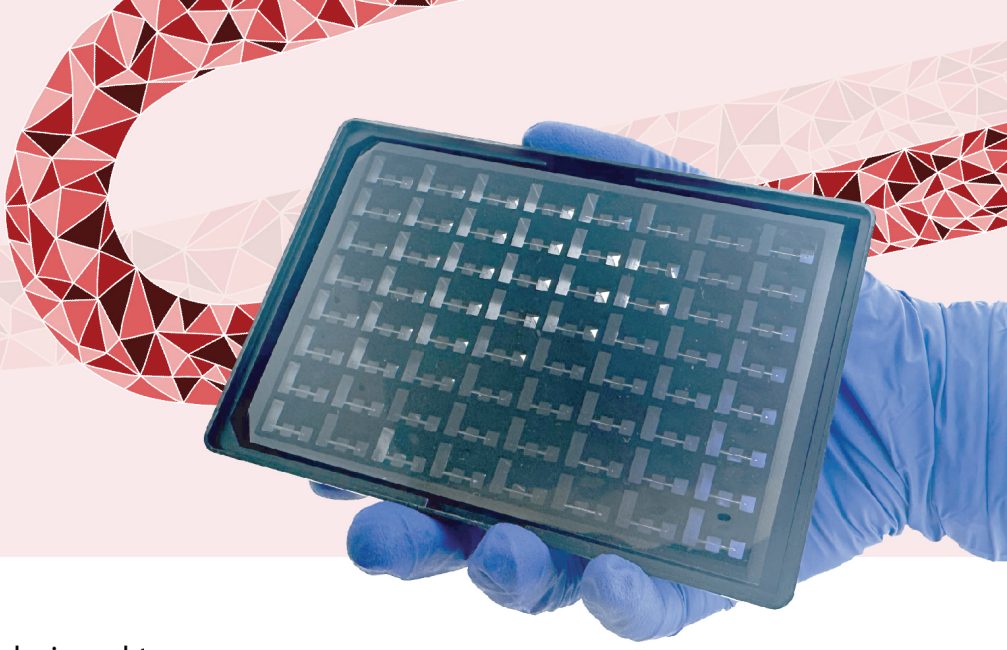
Inlet/outlet channel
0.5mm (W)
0.4mm (H)

Specifications

Product code	A002; A002-2; A002-3
Number of culture per plate	128
Sterilization method	UV sterilized
Storage condition	Room temperature (15-25 °C)
Storage time	3 month
Plate format	SBS standard 384 well plate
Materials	Top plate: virgin polystyrene. Bottom plate: optical quality, low compound-absorbing plastic Internal sacrifical template: proprietary polymers
Perfusion	Gravity driven perfusion with IFlowRocker (B001)
Applications	Perfused 3D tissue and organoid culture
Readouts	Sensor (TEER with AngioTEER, B002); Imaging (phase contrast, widefield fluorescence, confocal) ; plate reader (absorption, fluorescence, luminescence) ; off plate (Histology, ELISA, RNA/DNA analysis, MS, biochemistry)

Organo Biotech

UniPlate384™



UniPlate is an organ-on-a-chip platform designed to replicate the complex structure and function of human tissues under uni-directional media perfusion. Each UniPlate contains 64 independent tissue culture units, and is compatible with automation workflows. Each unit contains an inlet, middle, and outlet well connected together via two channels as well as a slanted ramp for media recirculation. The middle well contains a 3D-printed sacrificial template that can be encapsulated by a natural hydrogel. Upon dissolving the templates, the system produces a perfusable structure within the hydrogel matrix, which can then be populated with cells. The absence of membranes between the perfusable structure and surrounding matrix enables direct interactions between cells to mimick physiological tissue environments.

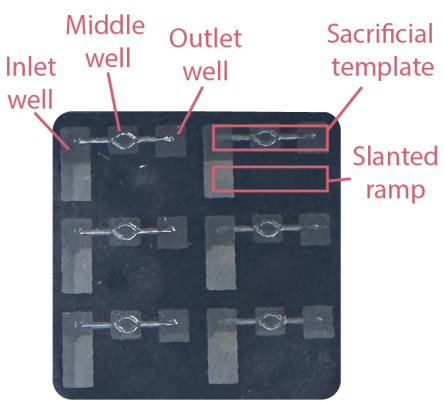
This versatile platform supports the creation of various tissue models, including tubular blood vessels, bifurcating vascular networks, renal epithelial tubules, colon tubes, and vascularized spheroids or organoids. These models can be used to study tissue barrier disruption, immune response, fibrosis and more in a highly controlled environment.

With customizable designs, including three standard templates and the option for tailored configurations, the UniPlate offers unparalleled flexibility for diverse research needs.

The UniPlate is specially designed to provide gravity-driven uni-directional perfusion of tissues when used in combination with the ***IFlowRocker***, eliminating the need for pumps. The UniPlate is also compatible with the ***AngioTEER*** sensor, enabling real-time, continuous monitoring of tissue barrier integrity across 64 tissue units in a plate. The open-well design ensures easy access for pipetting and tissue removal for downstream analysis, while the optically clear plate bottom supports high-content imaging using confocal microscopy and high-throughput analysis with plate readers. The 384-well design also minimizes the use of cells and reagents to significantly reduce experimental costs per data point. To learn more, please visit www.organo-biotech.com/posters.

KEY FEATURES

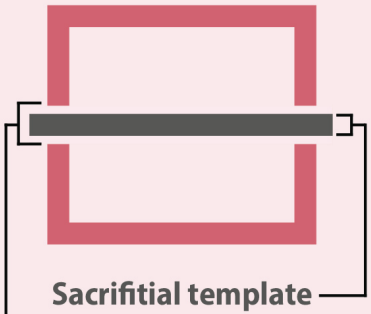
- 64 tissue culture units
- Pump-free uni-directional perfusion
- Long-term recirculation of immune cells
- Membrane-free 3D tissue culture in hydrogel matrix
- Customizable tissue architecture
- Automation compatible



Bottom view of the plate

Customizable tissue architecture

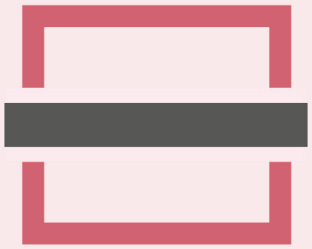
A003



Sacrifitial template
0.15-0.27mm (W)
0.05-0.1 mm (H)

Inlet/outlet channel
0.5mm (W)
0.4mm (H)

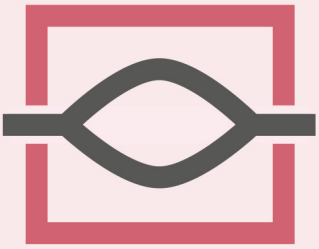
A003-2



Sacrifitial template
0.4-0.5mm (W)
0.2-0.3 mm (H)

Inlet/outlet channel
0.8mm (W)
0.5mm (H)

A003-3



Sacrifitial template
0.15-0.27mm (W)
0.05-0.1 mm (H)

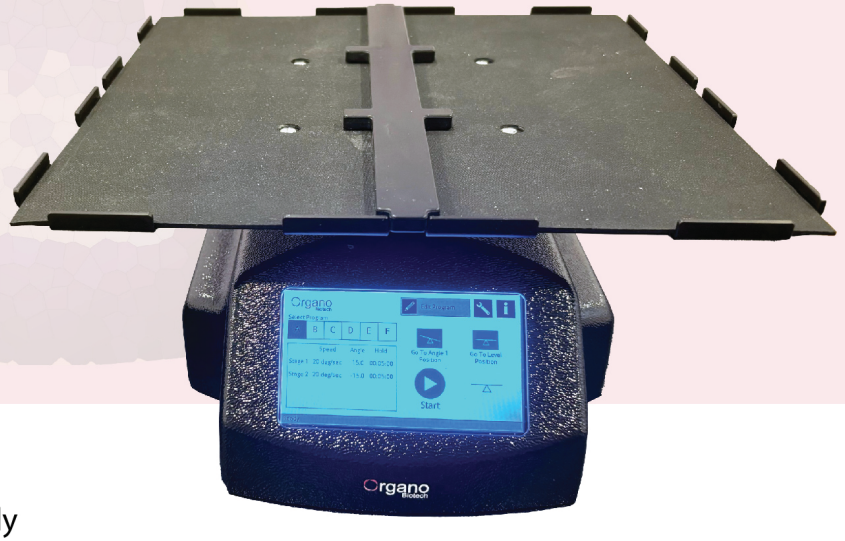
Inlet/outlet channel
0.5mm (W)
0.4mm (H)

Specifications

Product code	A003; A003-2; A003-3
Number of culture per plate	64
Sterilization method	UV sterilized
Storage condition	Room temperature (15-25 °C)
Storage time	3 month
Plate format	SBS standard 384 well plate
Materials	Top plate: virgin polystyrene. Bottom plate: optical quality, low compound-absorbing plastic Internal sacrifical template: proprietary polymers
Perfusion	Gravity driven perfusion with IFlowRocker (B001)
Applications	Perfused 3D tissue and organoid culture
Readouts	Sensor (TEER with AngioTEER, B002); Imaging (phase contrast, widefield fluorescence, confocal) ; plate reader (absorption, fluorescence, luminescence) ; off plate (Histology, ELISA, RNA/DNA analysis, MS, biochemistry)

Organo Biotech

IFlowRocker™



IFlowRocker is a programmable rocker specifically designed for the perfusion culture of ***IFlowPlate***, ***AngioPlate***, and ***UniPlate***. It supports bi-directional perfusion with IFlowPlate and AngioPlate, and uni-directional perfusion with UniPlate. Engineered for long-term use in CO_2 incubators, the rocker is compact, versatile, and easy to operate. Users can program speed, tilt angles, pause times, and other settings through an intuitive interface on a large graphical touchscreen.

This system enables gravity-driven perfusion at precise flow rates and durations, accommodating multiple culture plates simultaneously for high-throughput applications. When paired with UniPlate, the IFlowRocker delivers an *in vivo*-like unidirectional recirculating flow of both culture media and immune cells, representing the only commercially available system that offers gravity-driven unidirectional perfusion culture.

To learn more, please visit *www.organo-biotech.com/posters.*

KEY FEATURES

- **MIMIC IN VIVO CONDITIONS:**
  Set angles and pause times to control the flow of the media.
- **INCUBATOR SAFE:**
  Support long-term use in CO_2 cell culture incubators.
- **ROCK IT YOUR WAY:**
  Program any combination of angles, speeds and pause times, including assymetric rocking, for any flow conditions you want.
- **RELIABLE:**
  The rocker will run for years.
- **LARGE CAPACITY:**
  5 kg (11 pounds), on platform. Platform with optional stacking shelf (2nd level) can hold up to 12 plates.

Specifications

Product code	B001
Power requirement	24 VDC; 2.5 Amp
Operating relative humidity	5 – 90% non-condensing
Operating temperature	4 - 40°C
Altitude	**<2000m**
Storage temperature	-40 to 50°C
Programing capability	Continuous operation of a programmable cycle which includes: • Up to 6 programmable tilt angles (depending on the model). • Programmable speed to each tilt angle • Programmable pause can be set or bypassed for each angle, from 1 second to 90 minutes. • Tilt range up to ± 30 degrees. • Pause time from seconds to 1-1/2 hours • Uni-directional perfusion when used with UniPlate • Bi-directional perfusion when used with IFlowPlate/AngioPlate
Compatibility	Works with IFlowPlate, AngioPlate, and UniPlate,
Operational space requires	+\-30 degrees tilt with single shelf: minimal height of 20 cm; 37.5cm of width; and 39cm in depth
Weight of equipment	4kg
Load capacity	5kg, can hold 6 plates with optional stacking shelf (2nd level) that can hold additional 6 plates
Warranty	1 year
Certification	CE

Organo Biotech

AngioTEER™

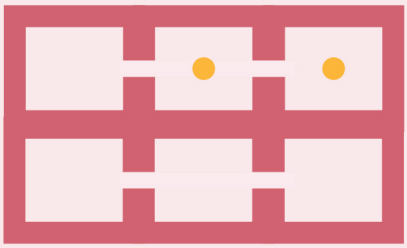
AngioTEER is a high-throughput and automated TEER meter developed to address the limitations of conventional TEER meters. Traditional TEER sensors lack high-throughput capabilities, creating challenges in measuring larger sample sizes. Additionally, their manual measurement process can introduce human bias and errors into the results. AngioTEER consists of two main components: an electrode board housing an array of gold-plated electrode pairs and an enclosure designed to encase the electrode board for user convenience. The electrode board includes a built-in power cable and Wi-Fi connection, enabling users to power and access the device seamlessly via Wi-Fi and the AngioTEER software, which automatically records measured values in real-time. The device is designed to be compatible with ***IFlowPlate***, ***AngioPlate***, and ***UniPlate***, and functions as a lid for seamless integration. AngioTEER enables automated, high-throughput evaluation of tissue barriers, particularly for assessing drug-induced toxicity in a time- and dose-dependent manner.

KEY FEATURES

- **BROAD AND ADJUSTIBLE RANGE:**
  Default range of 3,000–10,000 ohms, expandable to 50,000–200,000 ohms.
- **HIGH THROUGHPUT AND AUTOMATED DATA COLLECTION**
  Supports data collection from up to 128 tissues, with real-time continuous measurement of 64 tissues.
- **COMPACT AND EASY TO USE**
  Fully portable when powered by an external battery. Enables wireless data transfer to customized software for seamless data collection.
- **COMPATIBILITY**
  Compatible with IFlowPlate384 (A001-2), AngioPlate384, and UniPlate384.
- **DURABLE**
  Features easy-to-clean electrodes and a robust enclosure design for long-lasting performance.

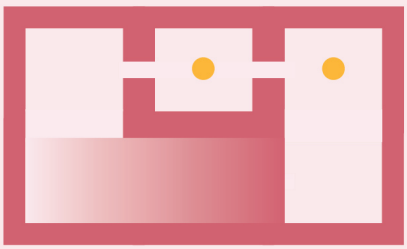
Customizable electrode positions

B002



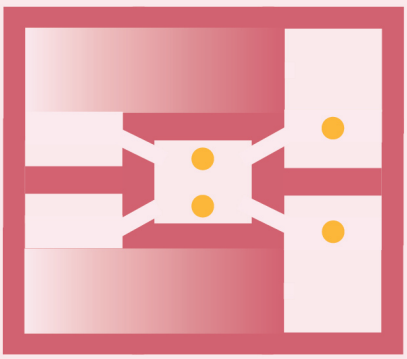
IFlowPlate384 (A001-2)
AngioPlate384

B002



UniPlate384

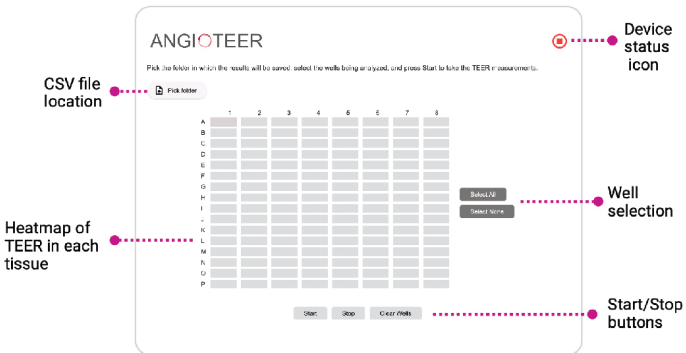
B002-2



UniPlate384 II

Specifications

Product code	B002; B002-2
Power requirement	24 VDC; 2.5 Amp
Operating relative humidity	5 – 90% non-condensing
Operating temperature	4 - 40°C
Altitude	<2000m
Storage temperature	-40 to 50°C
Compatibility	Works with IFlowPlate (A001-2), AngioPlate, UniPlate, and UniPlate II
Range	Default range of 3,000–10,000 ohms, expandable to 50,000–200,000 ohms.
Operational space requires	+\-30 degrees tilt with single shelf: minimal height of 20 cm; 37.5cm of width; and 39cm in depth
Weight of equipment	0.5kg
Warranty	1 year
Certification	CE
Software for data collection	



Organo Biotech

AeroLung™

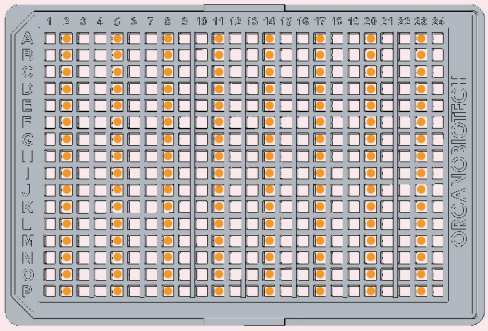
AeroLung is a system for uniform, high throughput aerosol delivery for the OrganoPlatforms and can expose up to 128 lung tissues at a time. It uses a pump and an air distributor for consistent deposition and offers programmable control for precise, spatially targeted dosing. AeroLung generates a fine aerosol by oscillating a piezoelectric mesh that atomizes any liquid formulation or particle suspension. A peristaltic pump then creates a steady downward airflow, driving the aerosols toward an air distributor that contains an array of nozzles that precisely align with the wells of an OrganoPlatform. Each nozzle has a fixed inner diameter and protrude into the wells to ensure a localized delivery of aerosol with controlled dose at the apical surface of the airway epithelial air-liquid interface. The device is customizable and compatible with ***IFlowPlate***, ***AngioPlate***, and ***UniPlate***. Together, AeroLung enables reproducible inhalation studies under near-physiological conditions.

KEY FEATURES

- **REPRODUCIBLE:**
  Even distribution of aerosols (CV<7%) across an entire IFlowPlate384.
- **HIGH THROUGHPUT:**
  Simultaneous aerosol delivery to 128 tissues
- **PROGRAMMABLE AND AUTOMATED:**
  Up to six programmable protocols for a variety of applications
- **LOCALIZED DELIVERY:**
  Spatially controlled delivery to apical side of epithelial barrier only
- **PRECISE DOSAGE:**
  Adjustable aerosol exposure dosages with exposure time and drug concentration
- **TARGETED DELIVERY AND CONCENTRATION GRADIENT GENERATION:**
  Spatially controlled delivery to selected wells and concentration gradient with customized air distributor or repeated exposure

Customizable air distributor design for gradient generation

B004



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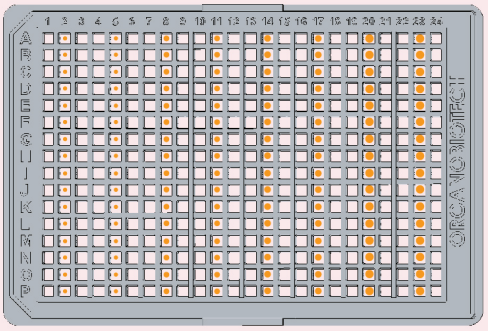
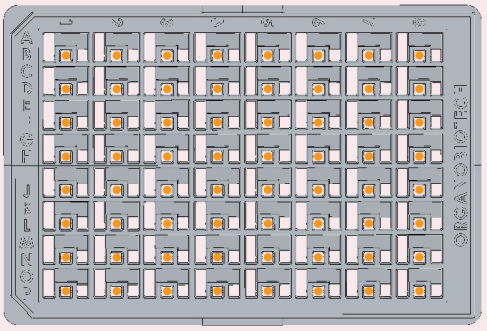


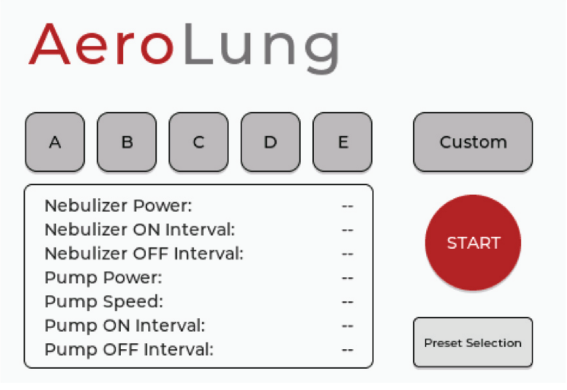
B004-3



- Air distributor nozzle position and size

Specifications

Product code	B004, B004-2
Power requirement	24 VDC; 2.5 Amp
Operating relative humidity	5 – 90% non-condensing
Operating temperature	20 - 30°C
Altitude	<2000m
Storage temperature	-40 to 50°C
Compatibility	Works with IFlowPlate, AngioPlate, UniPlate
Nebulizer parameter	108KHz (+\-3KHz), compatible with low viscosity fluid
Operational space requires	+\-30 degrees tilt with single shelf: minimal height of 20 cm; 37.5cm of width; and 39cm in depth
Weight of equipment	0.5kg
Warranty	1 year
Certification	CE
Programmable User Interface	--->



Automated and high-throughput production of blood vessel model for vascular toxicity screening and disease modeling

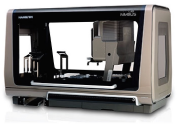
Dawn Song Yi Lin, Hanieh Hashemi, Anushree Chakravarty, Kimia Jozani, Jessica Bonanno, Nicky Anvari, Shravanthi Rajasekar, Feng Zhang, Boyang Zhang

Introduction

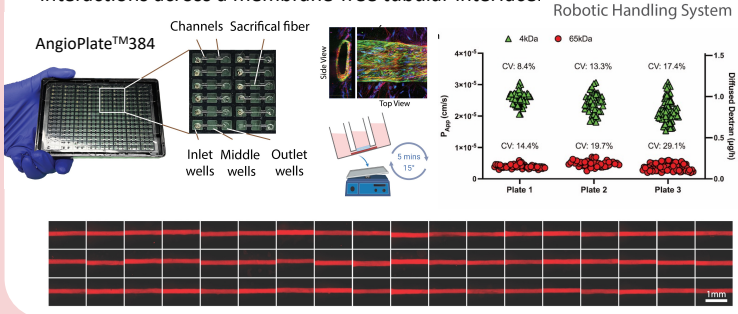
Vascular models are essential tools for research on vascular toxicity and disease mechanisms. However, traditional *in vitro* systems often fail to accurately recapitulate the structural and cellular complexity of native blood vessels. The ***AngioPlate384*** platform addresses this shortfall by enabling the scalable production of blood vessels from primary human cells with integrated stromal cell support, which help to provide key insights into cellular interactions, tissue responses, and pathophysiological processes. This poster outlines the principles behind the AngioPlate384 and its application in vascular model development.

AngioPlate™384 for blood vessel model

The production of a large array of 128 tubular blood vessels can be automated on AngioPlate384 to ensure consistent plate-to-plate reproducibility. These engineered vessels facilitate stromal-endothelial interactions across a membrane-free tubular interface.

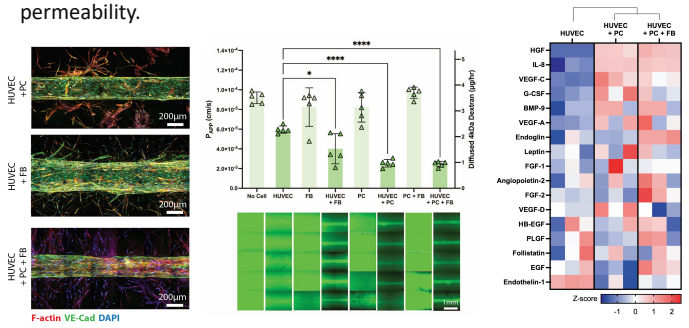


Robotic Handling System



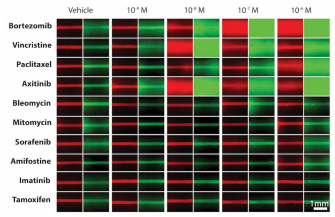
Stromal cells improve vascular barrier

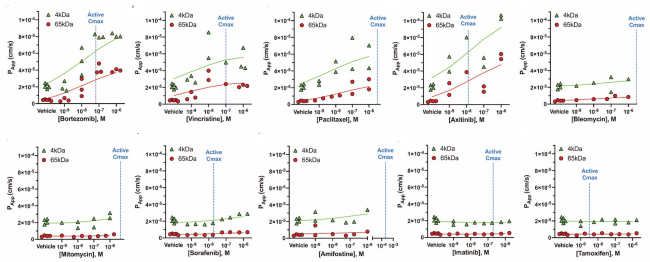
Stromal cells play a pivotal role in sustaining vascular integrity. Specifically, fibroblasts and pericytes produce hepatocyte growth factor (HGF), vascular endothelial growth factor-C (VEGF-C), and angiopoietin-2, which collectively contribute to a marked decrease in vascular permeability.



Drug screening for vascular toxicity

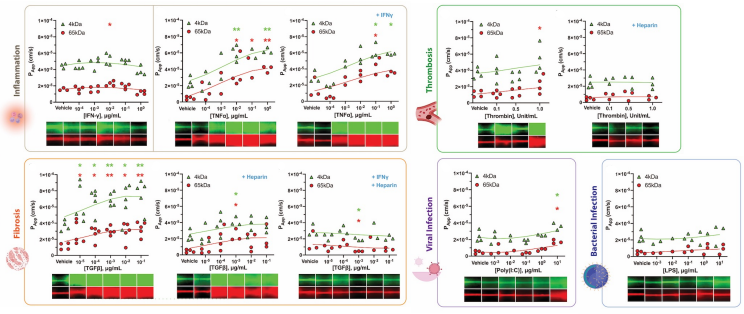
An analysis of vascular toxicity was conducted on ten clinical chemotherapeutic drugs administered at five varying doses. Within the dosages examined, four of these drugs demonstrated vascular toxicity.





Vascular injury

Changes in vascular barrier function due to various injuries and exposures can be examined through dose-response analysis. Findings reveal that tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) significantly compromise vascular integrity. Conversely, heparin and interferon-gamma (IFNγ) have been shown to modulate the effects of TGF-β.

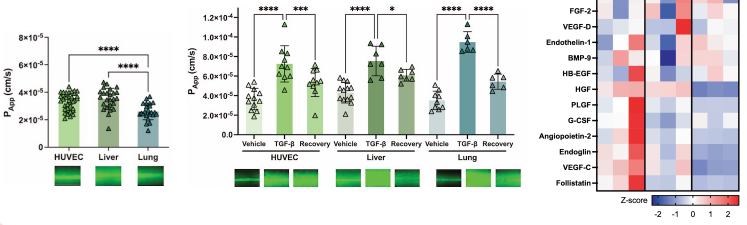


Conclusion

The AngioPlate384 platform provides robust and scalable production of tubular blood vessels with stromal cell support for toxicity screening, modeling vascular injury and diseases, and studying organ-specific response.

Organ-specific blood vessels

Blood vessels composed of organ-specific endothelial and stromal cells exhibit varied barrier permeability and reactions to TGF-β, along with differing recovery patterns. Specifically, liver blood vessels secrete minima HGF and demonstrate increased leakiness compared to those in the lung.



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Lin, D. S. Y., Hashemi, H., Jozani, K., Chakravarty, A., Bonanno, J., Anvari, N., Rajasekar, S., Zhang, F., Zhang, B. (2024). Automated and high-throughput production of blood vessel model for vascular toxicity screening and disease modeling. (Under review)

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High-throughput Platform for Modeling Tubular Injuries in Kidney

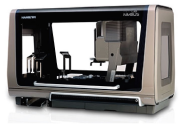
Shravanthi Rajasekar, Anushree Chakravarthy, Brenda Truong, Kimia Asadi, Matana Hendrickson, Ahmed Attia, Muna Sabouny, Anna Basatskaya, Madeline Ludlow Alexander Sotra, Dawn S. Y. Lin, Feng Zhang, Sergi Clotet-Freixas, and Boyang Zhang

Introduction

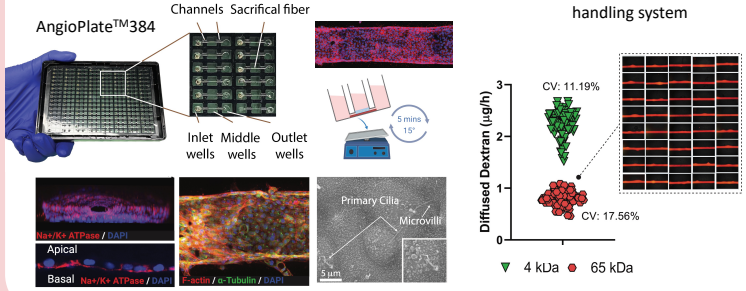
Renal tubular injury is a predominant cause of both acute and chronic kidney diseases. This damage often stems from exposure to nephrotoxins or ischemic events, which can lead to tubular interstitial fibrosis. Despite the clinical significance of these injuries, their pathophysiology is not fully understood. In this study, we introduce an engineered 3D perfusable model of renal proximal tubules on the AngioPlate platform. We successfully replicated cisplatin-induced tubular injury, kidney reperfusion injury and further expanded our model to simulate renal fibrosis by co-culturing fibroblasts with renal proximal tubules. Our findings underscore the potential of this renal model to enhance our understanding of renal disease mechanisms and explore new therapeutic strategies.

AngioPlate™384 for blood vessel model

We automated the production of a large array of 128 renal proximal tubules using AngioPlate384. These engineered tubules demonstrate high consistency and reproducibility. They establish a polarized barrier and express primary cilia, microvilli, and Na+/K+ ATPase.

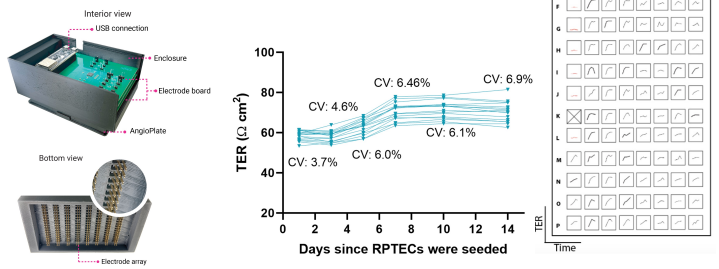


Robotic liquid handling system



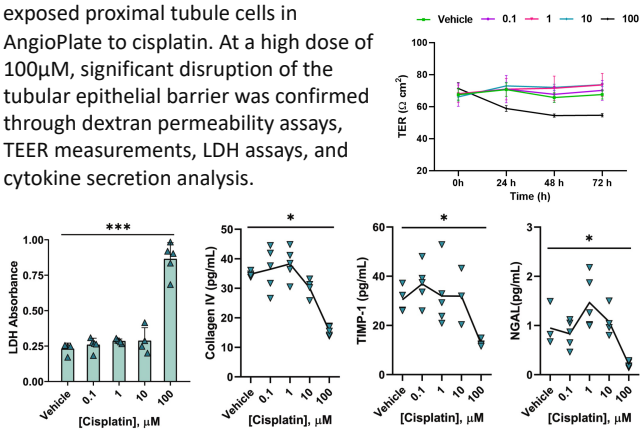
Tracking barrier formation with AngioTEER

We developed a non-invasive, automated Trans Electrical Epithelial Resistance (TEER) device named AngioTEER, which integrates with AngioPlate for real-time monitoring of tubular barrier integrity in both healthy and injured states. This device allows for consistent tracking of up to 128 tubules over time.



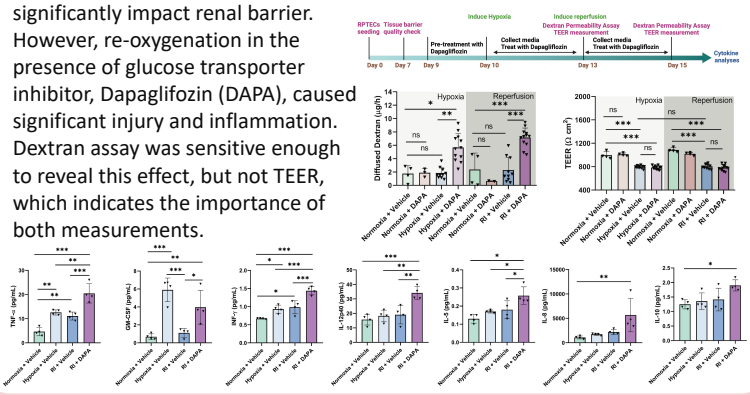
Renal drug toxicity testing

Cisplatin is employed to treat ovarian, testicular, and bladder cancers but induces renal injury in 20% of patients. To simulate this injury, we exposed proximal tubule cells in AngioPlate to cisplatin. At a high dose of 100µM, significant disruption of the tubular epithelial barrier was confirmed through dextran permeability assays, TEER measurements, LDH assays, and cytokine secretion analysis.



Reperfusion injury

Hypoxia and re-oxygenation induced TNF-α, GM-CSF, INF-γ secretion but didn't significantly impact renal barrier. However, re-oxygenation in the presence of glucose transporter inhibitor, Dapaglifozin (DAPA), caused significant injury and inflammation. Dextran assay was sensitive enough to reveal this effect, but not TEER, which indicates the importance of both measurements.

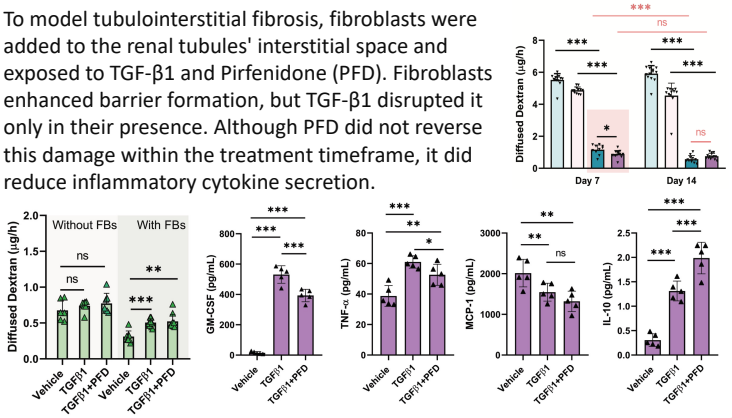


Conclusion

The AngioPlate384 platform provides robust and scalable production of kidney proximal tubules supported with interstitial fibroblasts for renal drug toxicity screening, reperfusion injury, and tubulointerstitial fibrosis.

Renal fibrosis

To model tubulointerstitial fibrosis, fibroblasts were added to the renal tubules' interstitial space and exposed to TGF-β1 and Pirfenidone (PFD). Fibroblasts enhanced barrier formation, but TGF-β1 disrupted it only in their presence. Although PFD did not reverse this damage within the treatment timeframe, it did reduce inflammatory cytokine secretion.



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Rajasekar, S., Chakravarthy, A., Truong, B., Asadi, K., Hendrickson, M., Attia, A., Sabouny, M., Basatskaya, A., Ludlow, M., Sotra, A., Lin, D. S. Y., Zhang, F., and Zhang, B. (2024). High-throughput Platform for Modeling Tubular Injuries in Kidney. (Under review)

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Cancer model with vascular recirculation reveals temporally dependent and tissue-specific macrophage recruitment

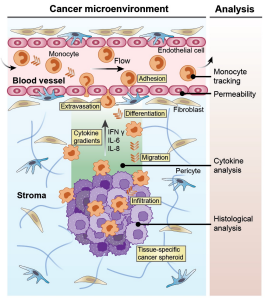
Feng Zhang, Kimia Asadi Jozani, Anushree Chakravart, Dawn Lin, Andrew Hollinger, Shravanthi Rajasekar, Boyang Zhang

Introduction

Recognizing the tumor-immune interaction being a dynamic and long-term process, we developed an immune-infiltrated cancer spheroid model by continuously perfusing and recirculating immune cells with gravity-driven flow through a tubular blood vessel adjacent to a cancer spheroid. Through continuous recirculation of monocytes, we successfully demonstrated the monocyte adhesion within blood vessels, transendothelium migration, differentiation and macrophage recruitment into cancer spheroids in breast carcinoma and hepatoma models over a week. The macrophage recruitment process was temporally dependent and tissue-specific. It naturally led to the formation of cancer-macrophage heterospheroids with a macrophage population that was continuously growing, renewing, self-evolving. This platform provides a valuable framework for investigating immune cell infiltration and differentiation within the tumor microenvironment, which is essential for the advancement of cancer immunotherapies.

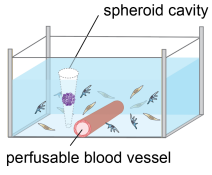
Recapitulating tumor microenvironment

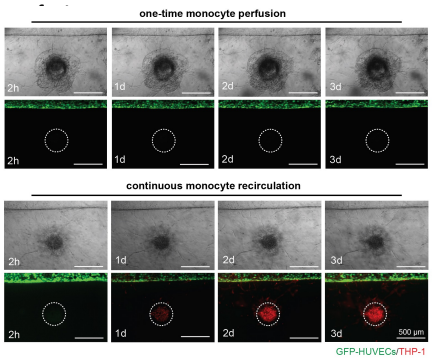
Recent advances in tumor modeling now incorporate immune components into the tumor microenvironment, recognizing the key role of immune cell recruitment and differentiation in tumor development. However, existing models face challenges in accurately simulating this complex interaction. One major obstacle is the ongoing recruitment of immune cells from circulation into the tumor environment, a process that differs markedly from the conventional one-time cell seeding approach commonly used in tissue engineering.

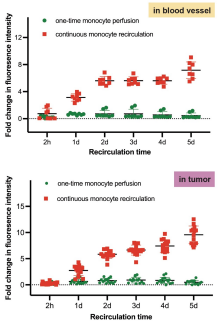


Continuous long-term recirculation of immune cells

Cancer spheroids can be precisely positioned adjacent to blood vessels. This setup, featuring continuous long-term recirculation, more accurately mimics the recruitment and accumulation of immune cells from circulation into cancer spheroids compared to one-pass



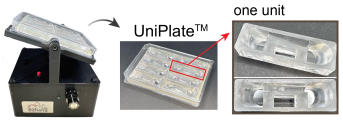


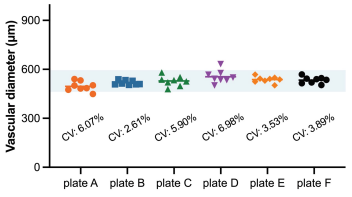


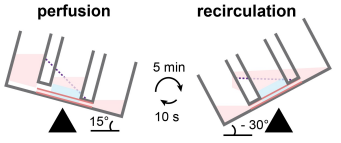
GFP-HUVECs/THP-1

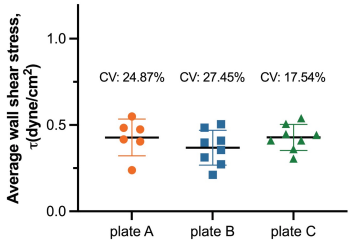
Blood vessel under unidirectional flow

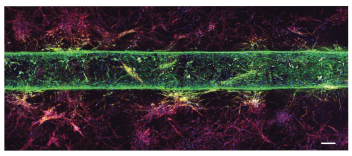
The UniPlate, used in conjunction with the IFlowRocker, provides continuous recirculating flow in a multi-well plate format and enable unidirectional perfusion of tubular blood vessels. This vascular model maintains consistent vessel diameters and flow parameters across different wells and plates.







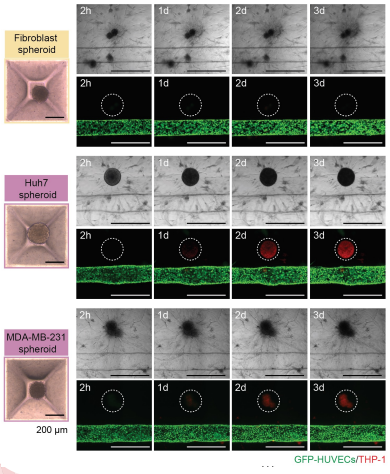


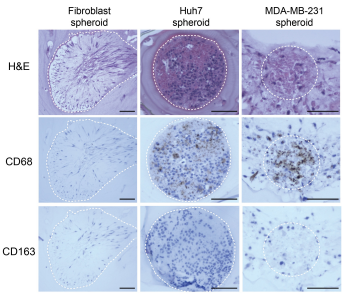


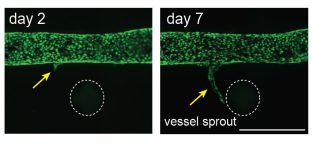
VE-Cadherin/F-actin/DAPI

Macrophage recruitment in tumor spheroids

Monocyte recruitment was observed only in the presence of cancer spheroids, not with non-cancerous fibroblast controls. The recruited monocytes transformed into CD68(+) and CD163(-) M1-like macrophages within the cancer spheroids. Additionally, vascular sprouting towards the cancer spheroids was noted following macrophage recruitment, highlighting the role of tumor-associated macrophages in remodeling the tumor microenvironment. The creation of heterotypical cancer spheroids with innate immune cells recruited from circulation could enhance our understanding of cancer immunotherapies.







GFP-HUVECs/THP-1

Conclusion

In summary, we developed immune-infiltrated cancer spheroid models that could be used to recapitulate the essential components of cancer microenvironment for applications in testing immunotherapeutic.

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References

Zhang, F., Jozani, K.A., Chakravarty, A., Lin, D., Hollinger, A., Rajasekar, S., & Zhang, B. (2024). Immune-infiltrated cancer spheroid model with vascular recirculation reveals temporally dependent and tissue-specific macrophage recruitment. (Under review)

High-Throughput Vascular Integration Using AngioPlate with Bifurcated Vasculature for Tissue Spheroids and Organoids

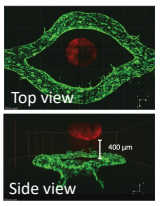
Andrew Hollinger, Wanqi Chen, Feng Zhang, Britney Tian, Mina Ogawa, Shinichiro Ogawa, Boyang Zhang

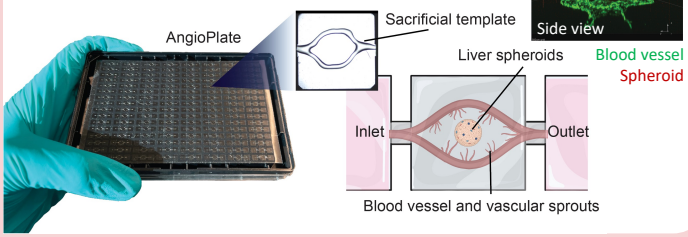
Introduction

Integrating perfusable vasculature with tissue spheroid cultures has traditionally been complex yet essential for enhancing tissue functionality[1]. In this study, we successfully demonstrate the formation of bifurcating vasculature within a fibrin matrix on an **AngioPlate384**[2]. We used a TopoStamp to embed a microwell into the fibrin gel. Huh7 liver spheroids and primary human liver organoids were then placed in the microwell, surrounded by perfusable blood vessels and supportive stromal cells. Interaction between the Huh7 liver spheroids and the vasculature increased vessel permeability. Additionally, the vascular perfusion enhanced albumin production from the primary human liver organoids. This platform may offer an improved culture environment that supports the sustained maintenance of primary liver functions *in vitro*, which is much needed for studying drug metabolism and toxicity screening.

AngioPlate bifurcated network to support spheroid integration

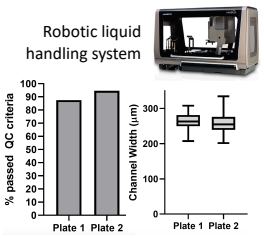
An AngioPlate with a 3D-printed sacrificial template featuring a bifurcated network design can be used to create perfusable blood vessel networks with robust vascular connections.

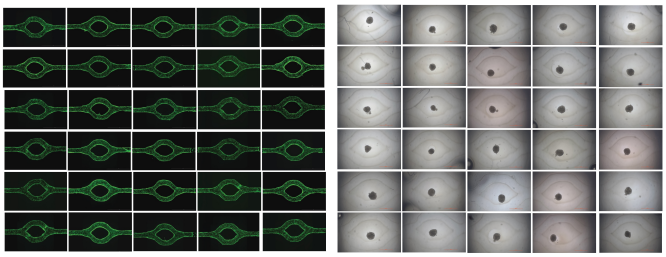




Robust transfer of tissue spheroids

The production of a large array of bifurcated blood vessel networks and the transfer of spheroids from a U-bottom plate to an AngioPlate can be automated using a robotic liquid handling system on the AngioPlate. A single spheroid can be precisely positioned in relation to the bifurcated network.





Conclusions

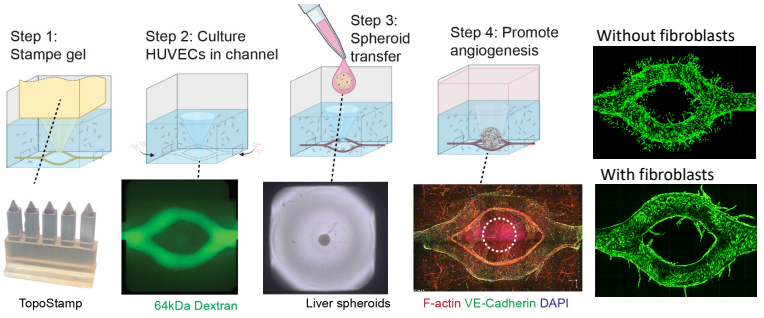
We have successfully demonstrated a new method using the AngioPlate to support the culture of 3D liver spheroids and organoids. This approach has the potential to be applied to other types of tissues, including vascular, cortical, and kidney organoids, where vascular perfusion is required.

References

1. Rajasekar, S. *et al. Adv. Mater.* **32**, 2002974 (2020)
2. Hollinger, A. *et al.* High-Throughput Vascular Integration Using AngioPlate with Bifurcated Vasculature for Tissue Spheroids and Organoids. (in preparation)

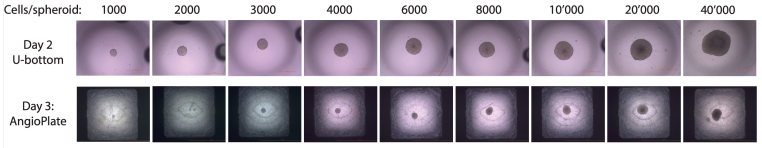
Improving tissue spheroid culture with perfusable bifurcated vasculature

A 3D-printed TopoStamp was used to introduce a microwell in the gel at the center of the vascular network. Spheroids can be cultured separately from the blood vessel and then combined on demand. Fibroblasts in the gel matrix help maintain organized vascular sprouting.



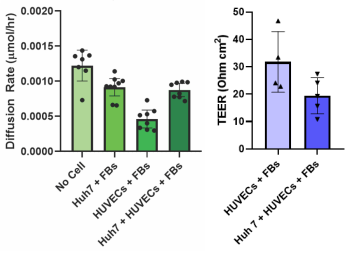
Accommodating spheroids of various sizes

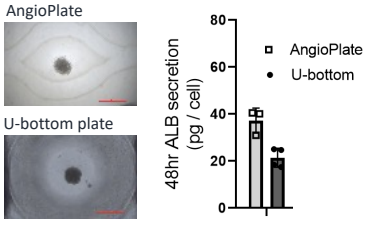
Spheroids of various sizes, ranging from 50 µm to 1 mm, can be produced by controlling the seeding cell number and then transferred to the AngioPlate.



Cross-talk between blood vessel, Huh7 spheroids, and primary liver organoids

In the presence of fibroblasts (FBs), endothelial cells (HUVEC) formed an effective barrier; however, this barrier became more permeable in the presence of Huh7 liver spheroids, as evidenced by both dextran diffusion assays and TEER measurements.





Albumin (ALB) production from primary liver organoids was significantly enhanced when cultured on AngioPlate with vascular perfusion, compared to static culture in commercial U-bottom plates.

Automated and high-throughput production of airway barrier model for modeling viral infection and asthma

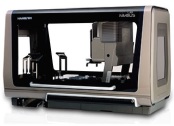
Kimia Asadi Jozani, Alexander Sotra, Nadia Milad, Karen Mossman, Matthew Miller, Manali Mukherjee, Jeremy Alexander Hirota, Boyang Zhang

Introduction

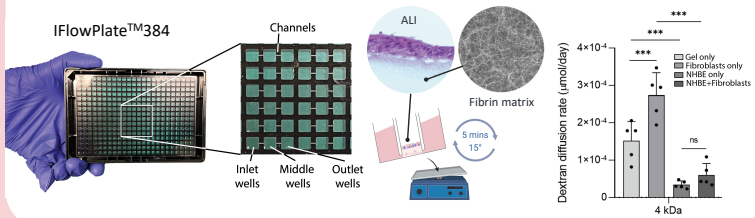
In vitro models of human airways are essential for understanding respiratory diseases and developing effective treatments. The IFlowPlate384 platform enables the automated, high-throughput production of differentiated primary airway models with air-liquid interface (ALI) critical for mimicking the natural human airway environment. We investigated the response of airway barriers to IL-13, simulating asthmatic conditions, and to influenza A viral infections. This approach allows us to examine the differential impacts on mucus secretion and the structural integrity of airway cells. The platform's precise control over cell culture conditions ensures reproducibility and scalability, making it invaluable for the screening of antiviral and anti-inflammatory therapies.

IFlowPlate™384 for airway barrier model

The automated production of a large array of airway barriers on the IFlowPlate384 ensures consistent reproducibility across different plates. Primary human airway cells were differentiated and matured on the IFlowPlate at an air-liquid interface (ALI) over a period of four weeks. During this time, the cells were continuously perfused, receiving nutrients from the basolateral compartment.

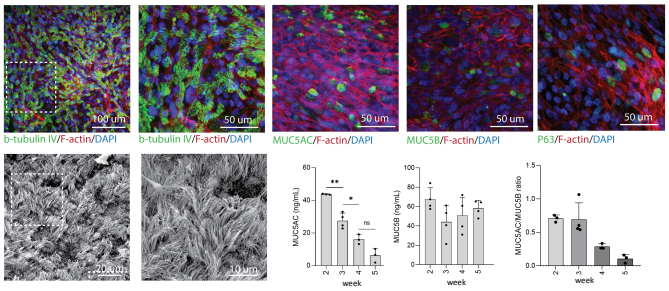


Robotic liquid handling system



Airway barrier with diverse cell population

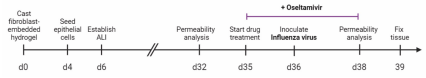
Primary human airway cells were successfully differentiated into β-tubulin IV (+) ciliated cells, MUC5AC (+) and MUC5B (+) goblet cells, and P63 (+) basal cells. The resulting cellular composition closely mirrors that of the human airway in vivo. Additionally, as the cells matured, a decrease in MUC5AC mucus secretion was observed.

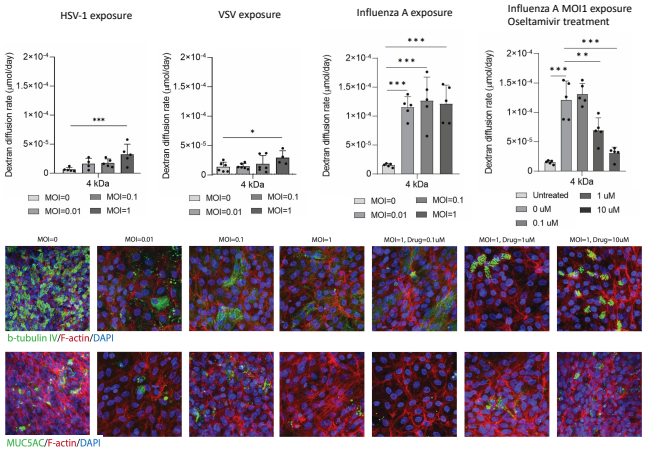


Viral infection and anti-viral drugs

Primary airway barriers, once differentiated, were infected with influenza A virus. These differentiated cells exhibit greater sensitivity to influenza A virus compared to the immortalized HBEC3-KT cell line. The antiviral drug oseltamivir demonstrated protective effects against the viral infection in a dose-dependent manner. Conversely, the airway barrier showed resistance to HSV-1 and VSV,

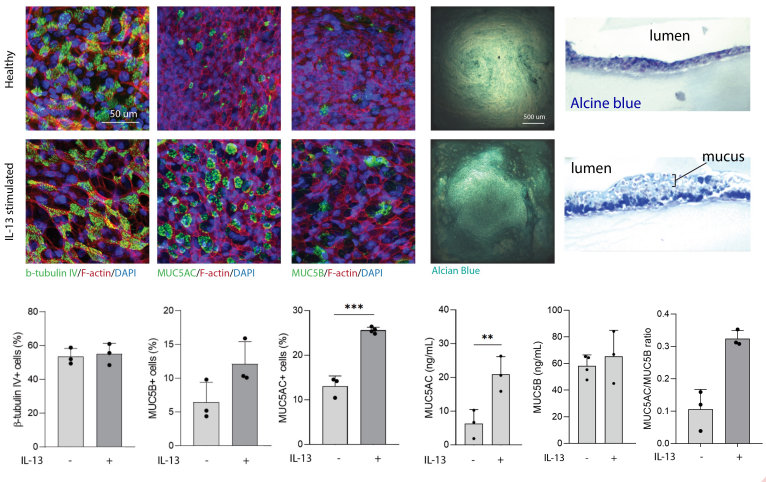
viruses that are not typically associated with human airway.





Asthmatic airway model

To simulate asthmatic conditions, airway barriers were exposed to IL-13, which induced a significant increase in mucus secretion, particularly from MUC5AC (+) mucin-producing cells. A thick mucus layer was observed at the airway ALI interface compared to healthy controls. IL-13 exposure did not significantly alter the function of ciliated cells or the secretion of MUC5B mucin.



Conclusion

The IFlowPlate384 platform provides an effective method for producing differentiated primary human airway models at scale with consistent reproducibility and control. Our studies demonstrate that the model closely mimics human airway responses to pathogenic infections and inflammatory stimuli and offers a valuable tool for advancing respiratory disease research and therapeutic development.

References

Asadi, K., Hirota, J. A., Zhang, B. (2024). Automated and high-throughput production of airway barrier model for modeling viral infection and asthma. (In preparation)

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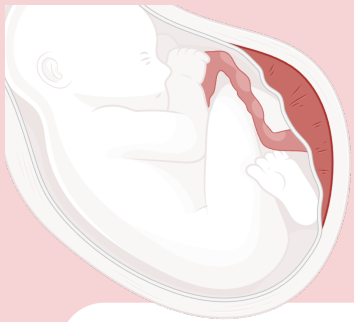


Human blastocyst-derived placenta barrier for drug transport studies

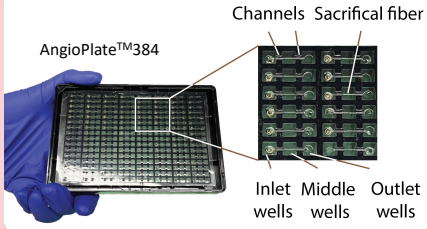
Sonya Kouthouridis, Madeleine Ludlow, Poonam Saha, Alexander Sotra, Zaim Khan, Justin Alvarado, Sandeep Raha, Boyang Zhang

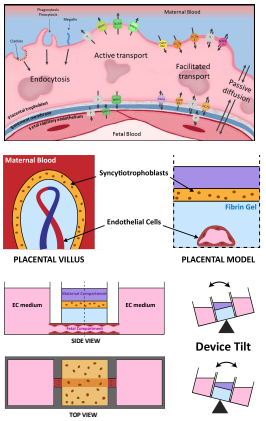
Introduction

Throughout pregnancy, the placental barrier is crucial for fetal development. Although the placenta has the capacity to selectively filters compounds, harmful xenobiotic substances from the maternal blood can sometimes cross over into the fetal circulation. This drives the development of in vitro placental barrier models in the context of drug transport studies. We developed a predictive model of placental transport using blastocyst-derived placental stem cells (PSCs) on AngioPlate384. We validate this model for drug barrier studies by assessing the permeability of three model therapeutic agents: paclitaxel, vancomycin, and IgG. Drug permeabilities were shown to be drug type, concentration, and size dependent, similar to what has previously been reported. Therefore, the presented model offers a promising tool for enhancing drug safety assessments in pregnant women, ensuring both maternal well-being and fetal health.

AngioPlate™384 for placenta barrier model

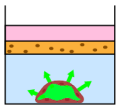
Late-stage placenta consists of vascularized chorionic villi encased in a thin, trophoblast layer, ideal for nutrient transport. To model this interface, a large array of placenta tissue barrier with both trophoblast and vascular interface can be produced on AngioPlate384 with high consistency.

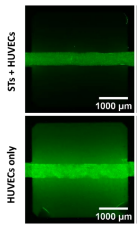


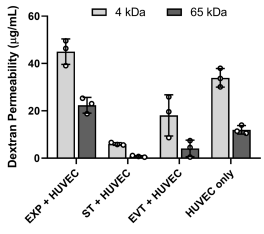


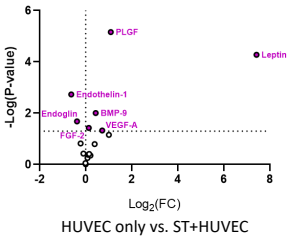
Syncytiotrophoblasts improved vascular barrier[2]

In co-culture conditions, the barrier formed by PSC-derived syncytiotrophoblasts (ST) significantly enhanced the vascular barrier compared to the endothelial-only control. The ST barrier secretes substantial levels of PLGF, leptin, and endothelin-1, among others, which are known to promote vascular health.









Conclusion

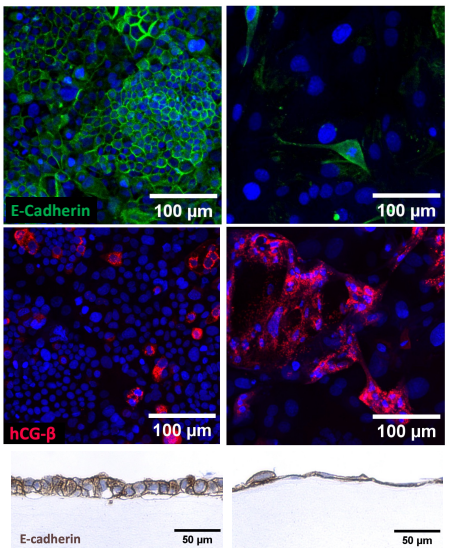
We have developed a 3D placental barrier model that integrates differentiated syncytiotrophoblast and endothelial layers, designed for compatibility with high-throughput testing. This model facilitates the assessment of drug permeability across various categories, including chemotherapeutics, antibiotics, and antibodies.

References

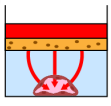
1. Kouthouridis, S. et al. Modeling the Progression of Placental Transport from Early- to Late-Stage Pregnancy by Tuning Trophoblast Differentiation and Vascularization. Advanced Healthcare Materials 12, 2301428 (2023).
2. Kouthouridis, S. et al. Human blastocyst-derived placenta barrier for drug transport studies (2024). Under Review

Human blastocyst-derived trophoblasts outperformed BeWo B30 line[1]

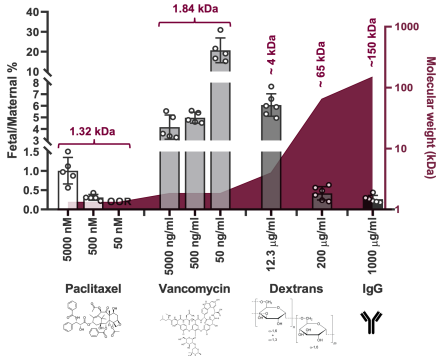
PSC differentiation results in a thinner, fused trophoblast layer, as well as an increase in human chorionic gonadotropin secretion, barrier permeability, and secretion of certain inflammatory cytokines, which are consistent with in vivo findings. Further, gene expression confirms this shift toward a differentiated trophoblast subtype. PSC outperform BeWo line in every aspect tested.



Drug transport across the complete placenta barrier[2]



Drug permeability across the placenta barrier is influenced by drug size, property, and concentration in the maternal compartment. The barrier model successfully predicted the differential transport rate of paclitaxel and vancomycin, similar to reported by ex vivo models.



F/M ratios

	Our model	Ex vivo
Paclitaxel	OOR - 1%	4.3 ± 2.2 %
Vancomycin	4.2 - 20.7%	9.6 ± 4.0 %
IgG	0.27%	0.46 ± 0.08 %

AngioTEER – Automated and high-throughput evaluation of tissue barriers on AngioPlate

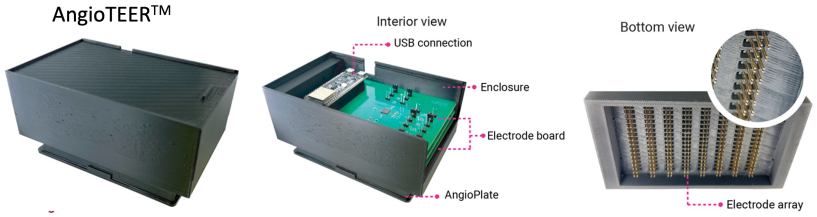
Shravanthi Rajasekar, Anushree Chakravarthy, Brenda Truong, Kimia Asadi, Matana Hendrickson, Ahmed Attia, Muna Sabouny, Anna Basatskaya, Madeline Ludlow Alexander Sotra, Dawn S. Y. Lin, Feng Zhang, and Boyang Zhang

Introduction

While dextran permeability assay can provide information on how effectively the barrier prevents the transport of molecules of specific sizes, Trans Epithelial Electrical Resistance (TEER) measurements can offer non-invasive monitoring across various time points during experiments. However, conventional TEER sensor lacks high throughput capabilities, posing challenges in measuring larger sample size. Moreover, their manual measurement process can introduce human bias and error into the results. To overcome these limitations of conventional TEER meter, we introduce **AngioTEER**, a high-throughput and automated TEER meter.

AngioTEER Setup

AngioTEER consists of two main components: an electrode board housing an array of gold-plated electrode pairs, and an enclosure designed to encase the electrode board for ease of use. The electrode board is equipped with a built-in USB power cable and Wi-Fi connection, enabling users to easily power and access the device through Wi-Fi connection and the AngioTEER software, which can automatically record the measured values in real-time. The entire device was designed to be compatible with AngioPlate and function as a lid for seamless integration.

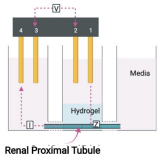


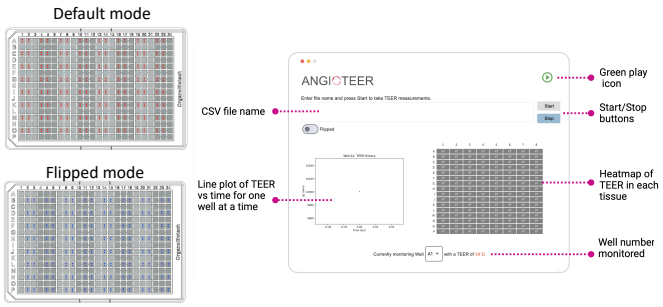
AngioTEER key features:

- Default detection range: 3000-10000 ohm
- Adjustable range: 50-200,000 ohm
- Data collection from 128 tissues
- Continuous measurement of 64 tissues
- Wireless data transfer
- Compatible with AngioPlate384, UniPlate384 (in development), and IFlowRocker

Electrode position and user interface

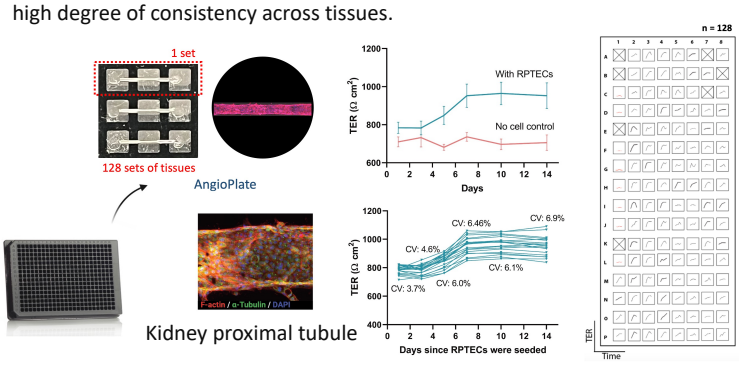
The electrodes are aligned such that, when the AngioTEER is mounted on the AngioPlate, one pair is inserted into the inlet well and the other into the tissue well. In this setup, one pair delivers a specific electrical current, while the other measures the resultant voltage to determine the total electrical resistance displayed on the screen. The placement of the electrodes, indicated by red and blue dots, can be alternated by rotating the AngioTEER 180°. This allows for measurements from all 128 tissue units by switching between the two modes.





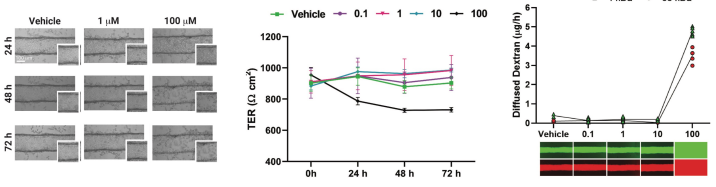
Tracking barrier formation of kidney proximal tubule over time in high-throughput

The functional barrier of up to 128 kidney proximal tubule tissue units can be assessed by measuring changes in electrical resistance. An increase in resistance indicates barrier formation, while a lack of significant change suggests the absence of a cellular barrier. The measurements demonstrate a high degree of consistency across tissues.



Detecting dose- and time- dependent cisplatin-induced nephrotoxicity in kidney tubule model

Drug-induced toxicity changes in the kidney proximal tubule barrier can be effectively detected using TEER and compared with dextran permeability measurements. TEER measurements highlight the time and dose-dependent nature of this toxicity.



Conclusion

The development of the AngioTEER enabled the automated evaluation of tissue barriers in high-throughput, particularly in the evaluation of drug-induced toxicity in a time and dose-dependent manner.

References

Rajasekar, S., Chakravarthy, A., Truong, B., Asadi, K., Hendrickson, M., Attia, A., Sabouny, M., Basatskaya, A., Ludlow, M., Sotra, A., Lin, D. S. Y., Zhang, F., and Zhang, B. (2024). High-throughput Platform for Modeling Tubular Injuries in Kidney. (Under review)

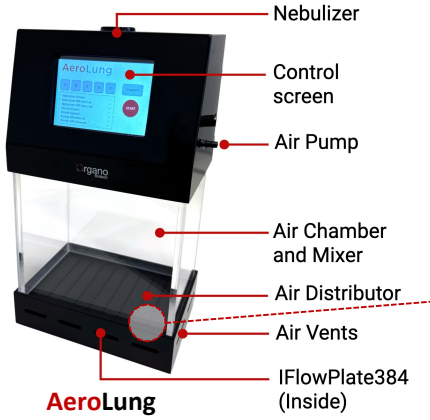
AeroLung – High-throughput *in vitro* exposure system for lung models on IFlowPlate384

Sara Deir, Manvir Bhangu, Justin Bernar, and Boyang Zhang

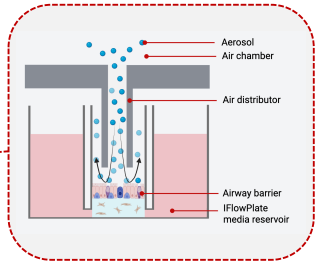
Introduction

Inhalation therapy targets the lung epithelium via aerosols, but most in vitro assays use submerged cultures, which are not physiologically accurate. Air-liquid interface models better mimic lung conditions, yet scalable and efficient exposure systems are limited. We developed ***AeroLung***, a system for uniform, high-throughput aerosol delivery across an ***IFlowPlate384***, exposing up to 128 lung tissues. It uses a pump and air distributor for consistent deposition and offers programmable control for precise, spatially targeted dosing. AeroLung enables reproducible inhalation studies under near-physiological conditions.

AeroLung Setup



AeroLung generates a fine aerosol by oscillating a piezoelectric mesh that atomizes any liquid formulation or particle suspension. A peristaltic pump then creates a steady downward airflow, driving the droplets toward a air distributor that contains an array of nozzles that precisely align with the wells of a IFlowPlate384. Each nozzle has a fixed inner diameter of 1mm and protrude into the IFlowPlate wells to ensure a localized delivery of aerosol with controlled dose at the apical surface of the airway epithelial air-liquid interface.



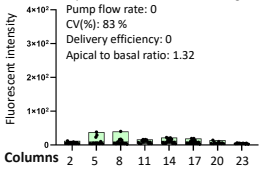
AeroLung key features

- Even distribution of aerosols across an entire IFlowPlate384
- Simultaneous aerosol delivery to 128 tissues
- Programable automated exposure protocol
- Spatially controlled delivery to apical side of epithelial barrier
- Adjustable aerosol exposure dosages
- Spatially controlled delivery to selected columns
- Aerosol concentration gradient across one IFlowPlate from single or multiple exposures

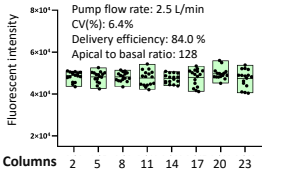
Static vs active exposure

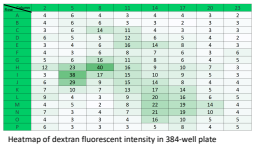
Airflow significantly improved aerosol delivery efficiency, uniformity (CV%), and apical delivery in active exposure.

Static exposure
Exposure duration: 2 minutes
Input dextran concentration: 6 mg/ml
Pump flow rate: 0
CV(%): 83 %
Delivery efficiency: 0
Apical to basal ratio: 1.32



Active exposure
Exposure duration: 2 minutes
Input dextran concentration: 6 mg/ml
Pump flow rate: 2.5 L/min
CV(%): 6.4%
Delivery efficiency: 84.0 %
Apical to basal ratio: 128

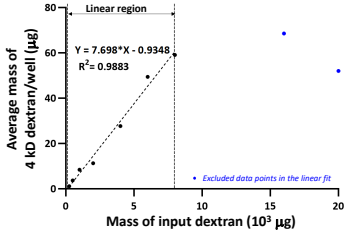




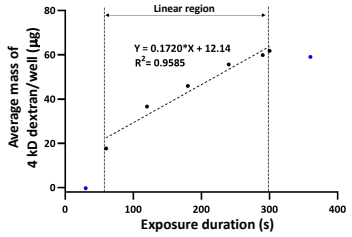
Heatmap of dextran fluorescent intensity in 384-well plate

Regulating aerosol exposure dosage

Dextran delivery to each well can be controlled by adjusting the dextran input concentration in a fixed 2-minute exposure.

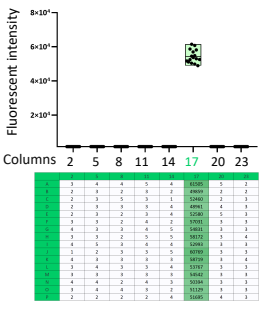


Dextran delivery to individual wells can be modulated by varying the exposure duration while maintaining a constant dextran input concentration of 8 mg/mL.

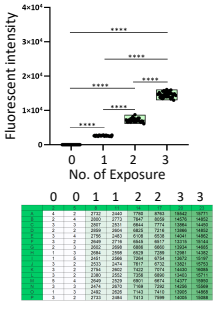


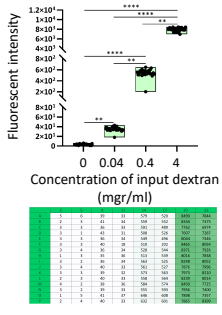
Regulating aerosol spatial delivery

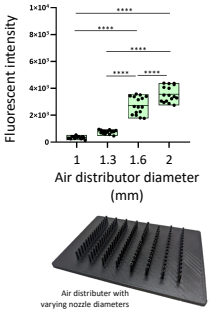
Customizable air distributer allowed targeted aerosol deposition to specific plate columns.



Dextran gradient was produced either by applying multiple exposures to selected columns, by changing the concentration of input dextran, or in a single exposure using an air distributor featuring nozzles of varying diameters.







Air distributer with varying nozzle diameters

Conclusion

Our results demonstrate that the AeroLung system enables uniform and efficient aerosol delivery across the high-throughput IFlowPlate384 platform, with precise control over exposure dose and spatial distribution. This system offers a practical solution for high-throughput inhalation drug screening applications.

Reference

[1] Lenz, Anke-Gabriele, et al., American journal of respiratory cell and molecular biology, 2014.

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