Application Note

Drug Discovery
and Development

Agilent
Trusted Answers

Making Barrier Integrity Assays Easier: Automated Imaging of Intestinal Barrier Function on a High-Throughput Organ-on-a-Chip Platform

Authors

Kristin Bircsak, Elena Naumovska, Henriette Lanz, Dwayne Dexter, Dimitri Pappaioannou, Anthony Saleh

MIMETAS US Inc.,

MIMETAS

Paul Held and Peter Banks
Agilent Technologies, Inc.

Abstract

This application note describes an efficient method for collecting and analyzing barrier integrity data with the MIMETAS OrganoPlate (r) and the Agilent BioTek Cytation cell imaging multimode reader, featuring rapid automated imaging of up to forty 3D cultures per plate.

Introduction

The intestinal barrier is critical to preventing systemic exposure to harmful substances. Disruption of enterocyte tight junctions by drugs or pathogens results in a compromised barrier function and increased absorption of potentially harmful intestinal contents (reviewed in 1).

In the drug development pipeline, early identification of permeability-inducing agents may help to better prioritize lead compounds and reduce the development of drugs that induce severe intestinal toxicity.[2]

Representative three-dimensional (3D) cell culture platforms including the MIMETAS OrganoPlate can be used early in the drug development process, offering a more accurate, cost-effective and time-efficient alternative to commonly used two-dimensional (2D) cell culture and animal studies.

Instrumentation

MIMETAS OrganoPlate

The OrganoPlate is a high-throughput organ-on-a-chip platform that provides a framework for culturing up to 96 individual tissue chips. Microfluidic channels connect the wells and allow for the addition of extracellular matrices (ECMs), cells, and media to build stratified tissues layer by layer. Short ridges called PhaseGuides separate the microfluidic channels, allowing for realistic, membrane-free coculture of multiple cell types. Blood flow is recapitulated in the OrganoPlate with a programmable rocker which uses gravity to drive the media between reservoir wells through the microfluidic channels. With these features, OrganoPlate models, including the gut-on-a-chip, mimic the structure and function of human tissues in vitro.

As previously described[3], the MIMETAS OrganoPlate platform incorporates 40 individual intestinal chips, which can be used to assess the effect of compounds on barrier function with fluorescent dyes. The high-throughput capabilities of the OrganoPlate allow for adequate sample sizes and reliable results. However, to appropriately compare the leakage of fluorescent dye between all 40 chips, the entire plate must be imaged in a short time.

This is cumbersome and nearly impossible to perform by hand with a standard fluorescence microscope. Therefore, a high-content imager, or automated microscope that can rapidly translate, focus, and image appropriate wells of the OrganoPlate, is required.

Agilent BioTek Cytation cell imaging multimode reader

The Agilent BioTek Cytation instrument line combines automated microscopy and conventional microplate detection in a configurable platform. The multimode detection modules include filter- and monochromator-based fluorescence detection, luminescence, and UV-Vis absorbance detection. The microscopy module offers up to 60x magnification in fluorescence, brightfield, high-contrast brightfield, color brightfield, and phase contrast. The Cytation C10 confocal imaging reader also supports spinning disk confocal and water immersion optics. Integrated environmental controls enable live-cell assays. Agilent BioTek Gen5 for imaging and microscopy software provides complete control over all imaging and data capture, plus image and data analysis. Luminescence detection can also be performed with the filter cubes either with or without emission filters.

The Gen5 software captures images, has extensive imaging processing and analysis capability. Among the analyses available are cell segmentation, cell counts, and subpopulation analysis based on cellular phenotypes.

Combining the platforms

This application note describes a method for assessing intestinal barrier function in the MIMETAS OrganoPlate with the Agilent BioTek Cytation 1 cell imaging multimode reader. Automated fluorescent imaging of 40 individual 3D cultures demonstrates an efficient method for collecting and analyzing barrier integrity data.

Experimental

Cells

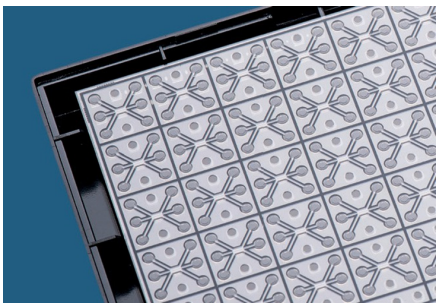
Caco-2 (MilliporeSigma), a human colon adenocarcinoma cell line, was used to prepare the intestinal model.

The OrganoPlate 3-lane 40

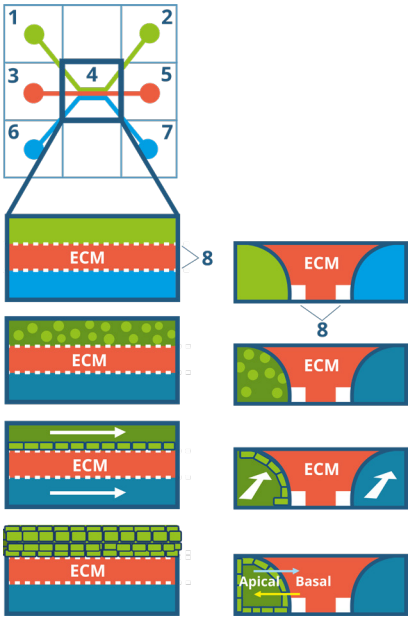
The top of the OrganoPlate is a 384-well microplate, while the bottom has been replaced with two layers of coverslip-quality glass. Microfluidic channels are embedded in between these two layers. For all experiments, an OrganoPlate 3-lane 40 (MIMETAS BV, 4003 400B) containing 40 individual tissue chips was used (Figure 1A). Each tissue chip is comprised of 9 wells (3 x 3), connected by three microfluidic channels (w: 400 µm, h: 220 µm), with inlets and outlets to access the cultures (Figure 1B). The three channels run parallel to each other in the center well, or observation window, and are separated by ridges called PhaseGuides.

Because of the glass bottom, the 3D OrganoPlate cultures can be viewed and imaged by microscopy through each observation window.

A.



B.



C.

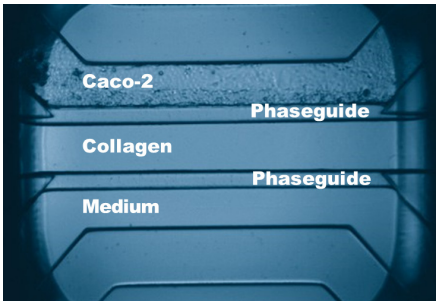


Figure 1. Establishing Caco-2 tubule cultures in the OrganoPlate 3-lane 40. (A) The modified bottom of a standard 384-well microplate with microfluidic channels embedded between glass plates. (B) Seeding Caco-2 against collagen I ECM: collagen I was added to the ECM inlet 3 to fill the middle channel. Once solidified, Caco-2 cells were seeded in the media inlet 1 to fill the top media channel. Through gravity, cells attached to the ECM, and after inducing flow, the Caco-2 cells form a tubular structure. (C) After five days in culture, a confluent monolayer is observed by brightfield microscopy using the Agilent BioTek Cytation cell imaging multimode reader.

OrganoPlate culture

A collagen I ECM (4 mg/mL) was seeded in the middle channel and allowed to solidify before seeding Caco-2 cells and media alone in media channels 1 and 2, respectively.[3] Once the cells adhered to the ECM, the OrganoPlate was placed on a rocking platform in the incubator (37 °C, 5% CO_2) to establish perfusion. After a few days in culture, a 2D brightfield image taken using the Cytation 1 cell imaging multimode reader demonstrated a confluent monolayer on the bottom of the microfluidic media channel (Figure 1C). Following immunofluorescent staining, images captured at multiple Z-positions within the channel can be combined to generate a 3D rendering that reveals a confluent tubule structure, with cells lining the ECM and all walls of the microfluidic channel (Figure 2).[3]

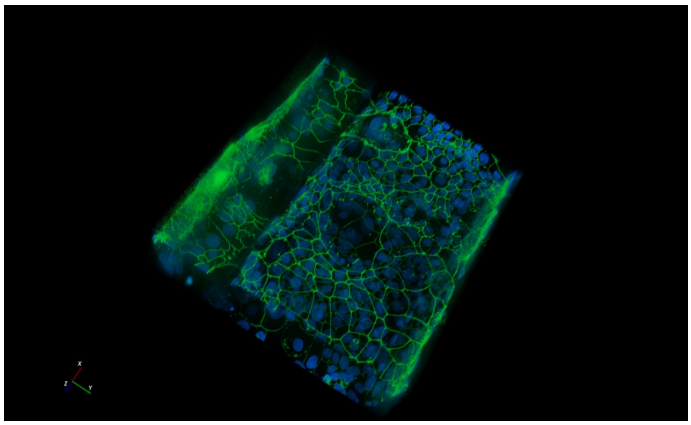


Figure 2. 3D rendering of Caco-2 OrganoPlate culture. Confocal fluorescence images were acquired using the Agilent BioTek Cytation C10 confocal imaging reader and 40x objective. A 3x3 image montage was collected at multiple z heights at 1 µm increments, spanning 150 µm Z-height range. Cell nuclei were labeled with Hoechst 33342 and cell membranes were labeled with GFP. 3D rendering was generated using the 3D Viewer tool in the Agilent BioTek Gen5 software.

Barrier integrity assay and automated imaging

To assess Caco-2 barrier function (Figure 3B), on day 6 of culture, media containing fluorescent dyes (FITC-Dextran 150 kDa, TRITC-Dextran 4.4 kDa; MilliporeSigma) and staurosporine (0 to 50 µM) were added to media channel 1 and imaging using the Cytation 1 (Figure 3A) began immediately. The Cytation 1 internal temperature was maintained at 37 °C and two fluorescent 2.5x images (Ex:Em 469:525, GFP; Ex:Em 531:593, RFP) were captured of every OrganoPlate chip for a total of 24 hours using 1 hour increments. Should barrier function be intact, the fluorescent dyes are retained within the tubule and absent from the ECM channel over time.

However, when the epithelial tight junctions are disrupted, the fluorescent dye will leak into the ECM channel (Figures 3C and D).

Data analysis

Image analysis was accomplished using the Gen5 image statistics function in conjunction with a rectangular plug to select the region of analysis. Independent regions of the image that correspond to the media (tubule lumen) and gel regions of the OrganoPlate channels were defined in the software. Image statistics automatically provide the mean fluorescence (GFP and RFP) of the defined regions only. The leakage score, defined as the ratio of the gel channel fluorescence divided by the media channel, was plotted for each time point.

Results and discussion

Caco-2 cells cultured in an OrganoPlate 3-lane 40 were treated with staurosporine (0 to 50 µM, 24 hours) and barrier integrity was assessed using the Cytation 1.

No cell and 0 µM staurosporine conditions were used as controls and exhibited the expected images and resultant leakage scores close to 1 and 0, respectively (Figures 4, 5, and 6A).

In the staurosporine treated cultures, there was a dose- and time-dependent loss of barrier integrity by leakage of both the 150 kDa FITC-Dextran and 4.4 kDa TRITC-dextran dyes from the media to the gel channel (Figures 4 and 5).

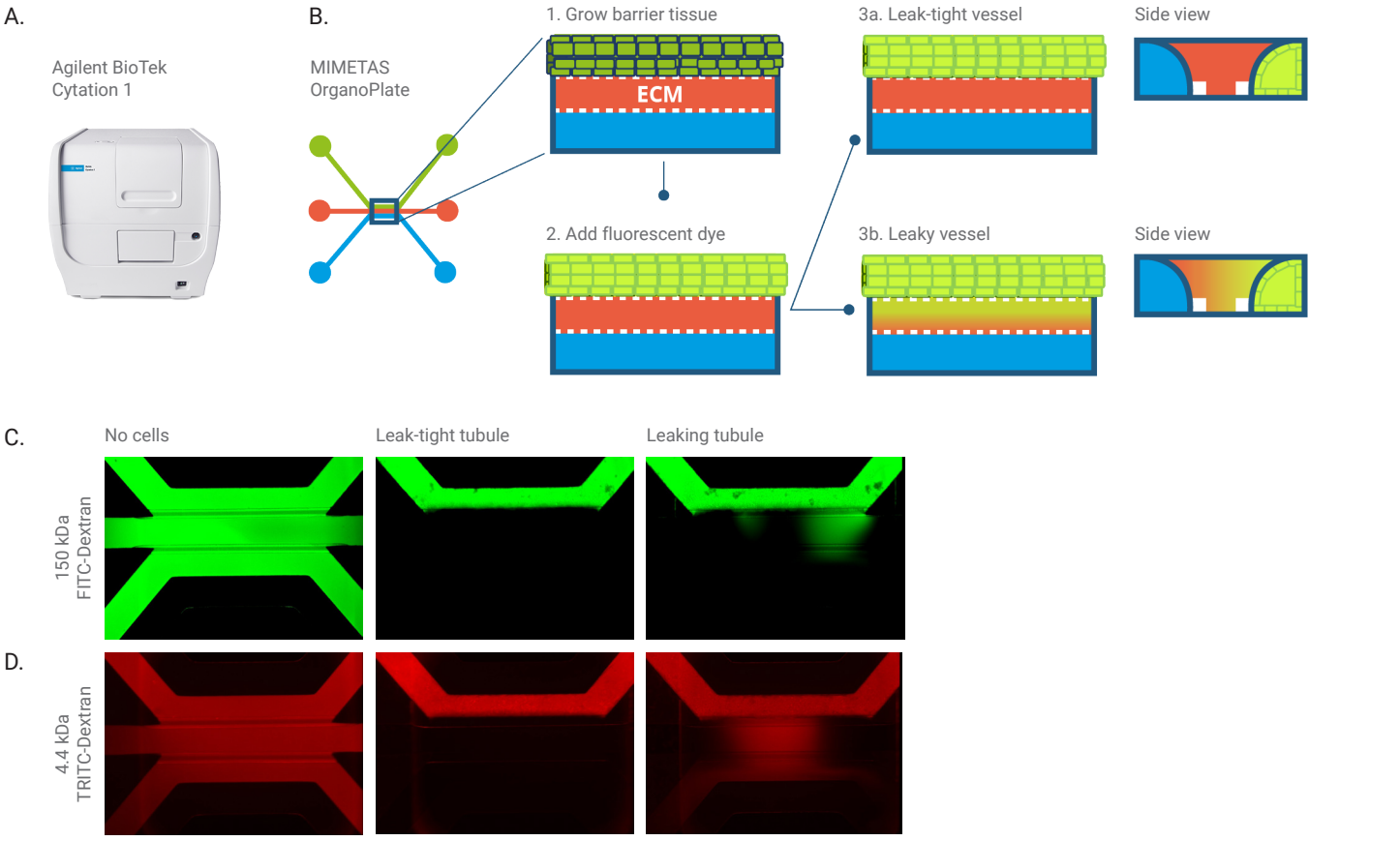


Figure 3. A barrier integrity assay using the Agilent BioTek Cytation 1 cell imaging multimode reader. (A) Using the Cytation 1, we performed the barrier integrity assay with the Caco-2 OrganoPlate cultures. (B) A schematic of the barrier integrity assay on a tubular culture in the OrganoPlate 3-lane 40 platform. The barrier integrity of the cell layer against the ECM was examined by perfusing the lumen of the tubule with dextran dyes. (C, D) In healthy, leak-tight tubules, dyes remain in the lumen, whereas in leaking tubules the dye enters the ECM channel.

From the entire dataset, both leakage score and apparent permeability were calculated and revealed that the 4.4 kDa TRITC-dextran (Papp=1.13 x 107 cm/s) leaked to a greater extent than the 150 kDa FITC-Dextran (Papp=0.612 x 107 cm/s) at a lower concentration of staurosporine (0.5 µM) (Figure 6B).

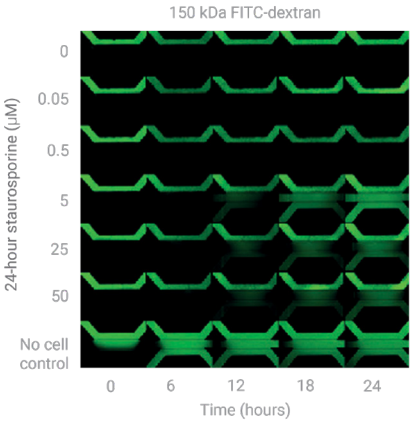


Figure 4. Permeability of FITC-dextran (150 kDa) in Caco-2 tubules treated with staurosporine. On day 6, Caco-2 tubules were treated with staurosporine (0 to 50 µM) and FITC-dextran (150 kDa), and barrier function was assessed. Dye leakage into the collagen I ECM was monitored every hour for a total of 24 hours using the Agilent BioTek Cytation 1 cell imaging multimode reader.

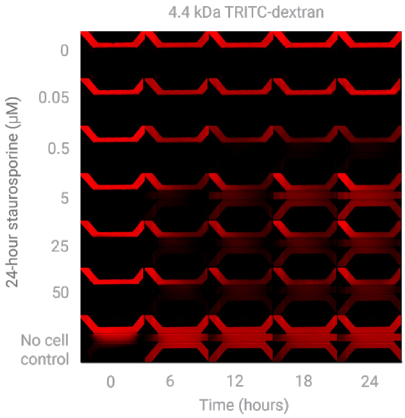


Figure 5. Permeability of TRITC-dextran (4.4 kDa) in Caco-2 tubules treated with staurosporine. On day 6, Caco-2 tubules were treated with staurosporine (0 to 50 µM) and TRITC-dextran (4.4 kDa) and barrier function was assessed. Dye leakage into the collagen I ECM was monitored every hour for a total of 24 hours using the Agilent BioTek Cytation 1 cell imaging multimode reader.

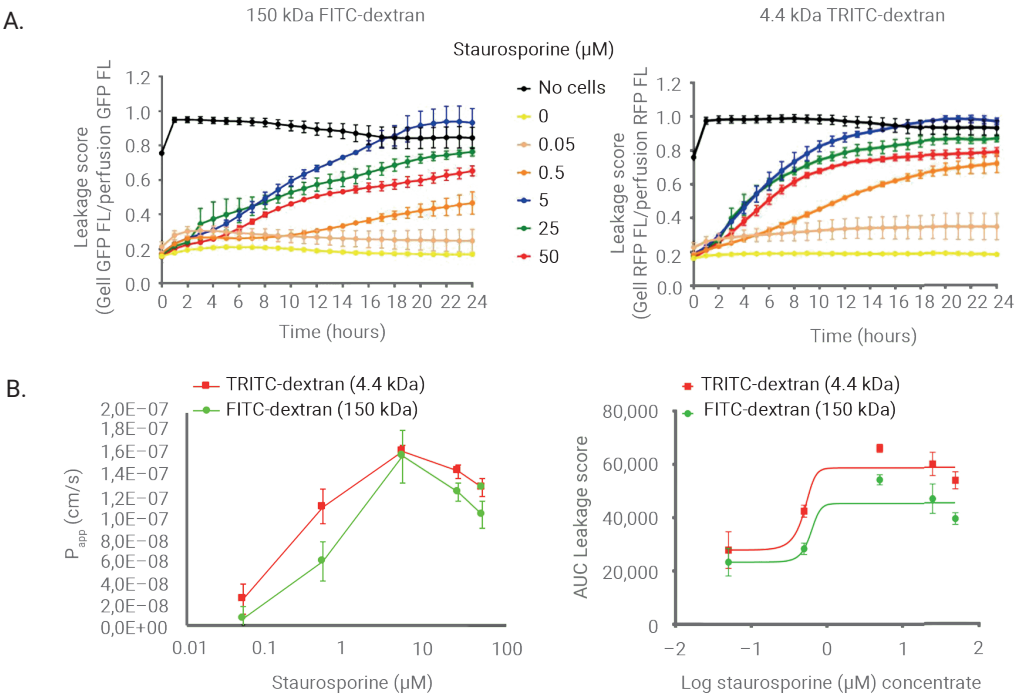


Figure 6. Barrier integrity data analysis and apparent permeability. (A) Leakage score was calculated by dividing the fluorescence intensity of the gel channel by the fluorescence intensity of the perfusion channel. A number close to 1 represents a leaky tubule, or no cells, while a number close to 0 represents a leak-tight tubule. (B) Apparent permeability (P_{app}, cm/s) was calculated by dividing the difference in fluorescence intensity between the gel (arbitrary units) and perfusion channel by the total time of the experiment (seconds), multiplied by the volume of the gel (cm^3), divided by the surface area of the barrier (cm^2), with an increase in P_{app} representing dye leakage.

Conclusion

The Agilent BioTek Cytation 1 cell imaging multimode reader and the Agilent BioTek Gen5 software for imaging and microscopy were used to assess the barrier integrity of 3D intestinal tissues grown in the MIMETAS OrganoPlate. Together, the two platforms offer a user-friendly interface, enabling a rapid and efficient method for data collection and analysis.

References

1. König, J. et al. Human Intestinal Barrier Function in Health and Disease. *Clin Transl Gastroenterol.* **2016**, *7*, e196. DOI: 10.1038/ctg.2016.54
2. Bowes, J. et al. Reducing Safety-Related Drug Attrition: The Use of In Vitro Pharmacological Profiling. *Nat Rev Drug Discov.* **2012**, *11*, 909–922. DOI: 10.1038/nrd3845
3. Trietsch, S. J. et al. Membrane-Free Culture and Real-Time Barrier Integrity Assessment of Perfused Intestinal Epithelium Tubes. *Nat Commun.* **2017**, *8*, 262. DOI: 10.1038/s41467-017-00259-3
4. Johnson, D. How to Do Everything: Digital Camera [Online], *McGraw Hill Professional*. **2008**.
5. Ray, S. F. Applied Photographic Optics, 3rd Edition, *Focal Press*. **2002**.

Products used in this application

Agilent BioTek Cytation 1 cell imaging multimode reader
Agilent BioTek Gen5 software for imaging and microscopy
MIMETAS OrganoPlate

www.agilent.com/lifesciences/biotek

RA45632.6463425926

Published in the USA, November 18, 2024
5994-7647EN

Agilent
Trusted Answers