APPLICATION NOTE

Automated Transepithelial Electrical Resistance (TEER) Measurements Allow for Rapid Screening of the Gastrointestinal Toxicity Profile of Therapeutics

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Abstract

Gastrointestinal toxicity (GIT) ranks among the most common clinical side effects for many orally administered pharmaceuticals, in particular for oncology drugs which are administered over an extended time period. Previously, we utilized an *in vitro* small intestine (SMI) microphysiological tissue model, EpiIntestinal™ (MatTek Corporation), which contains the epithelial cell types resident in the native human gastrointestinal tract, and transepithelial electrical resistance (TEER) measurements to evaluate the GIT profile of therapeutics and to predict oncology drug-induced diarrhea[1]. TEER is a non-invasive technique commonly used to monitor barrier integrity in 2D monolayers and 3D tissues. Daily TEER measurements were made over a six-week period, a typical therapeutic timeframe for oncology drugs, to monitor barrier integrity of 3D EpiIntestinal™ tissues grown on permeable membranes in 96-well insert plates. Manual TEER measurements were made on one tissue at a time, requiring 35-40 minutes to complete measurements for all 96 tissues. This manual method of measuring TEER limited the throughput of drug screening capabilities of the assay method, which is critical for the application of microphysiological systems in drug discovery and preclinical development. To overcome this challenge, we utilized the EVOM™ Auto instrument (World Precision Instruments), a high-throughput TEER measurement system, which can accurately and reproducibly measure the TEER of a 96-well plate in 4 minutes and 10 seconds. The EVOM™ Auto can be housed within a biological incubator or biological safety cabinet and run using a scheduled cycle mode, an additional feature that improves the ease of use. The EVOM™ Auto significantly increases the screening throughput, avoids technician fatigue, and increases the accuracy of TEER measurements by automating consistent measurement locations within the well. In this study, we investigated the ability of chemicals which cause short-term (ethylene glycol tetra-acetic acid (EGTA)) and long-term (dextran sulfate sodium salt (DSS)) reversible barrier disruption in the EpiIntestinal™ model and demonstrated a controllable and reversible effect on barrier function as evaluated by automated TEER measurements and a Lucifer yellow permeability assay. These results demonstrate the applicability of the EVOM™ Auto to monitor and evaluate the SMI microphysiological tissue model for drug and chemically induced GIT and barrier phenotypes as seen in diseases like Crohn's Disease and Colitis. Implementing the EVOM™ Auto into this workflow enables high-throughput TEER measurements, resulting in rapid and reproducible readouts of barrier integrity for drug screening applications.

Methods

EpiIntestinal™ Tissue Preparation

Small intestine (SMI) epithelial cells were harvested from post-mortem donors following IRB approval. SMI cells were seeded onto PermaCell™ high pore density (0.4µm, $1.0x10^8$ pores/cm^2) PET membrane cell culture 96-well plates (MatTek Life Sciences; Ashland, MA), raised to the air liquid interface (ALI) and cultured in specially formulated culture medium designed to induce differentiation for two weeks. An image of the PermaCell™ 96-well system and a representative Hematoxylin and Eosin (H&E) stained cross-section of the EpiIntestinal™ tissue model are shown in Fig. 1A-D.

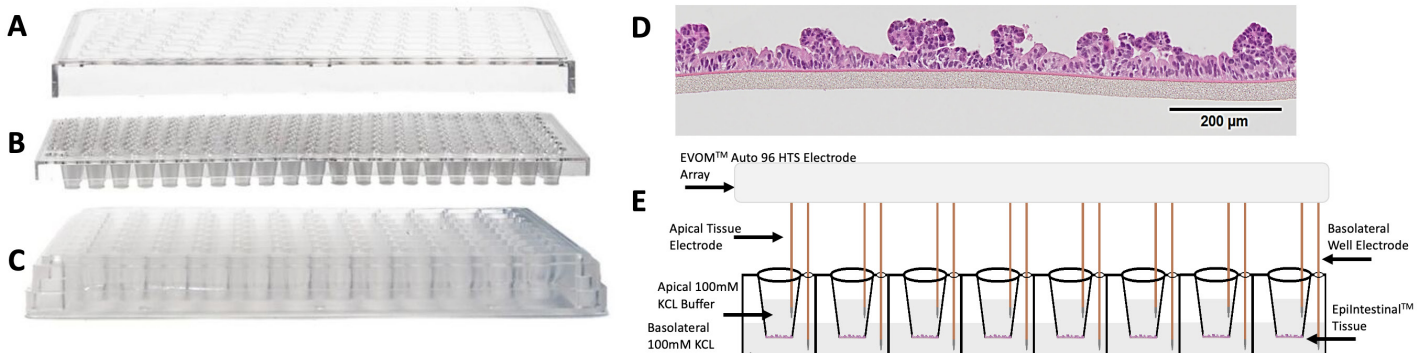


Fig. 1—Measurement of TEER on EpiIntestinal tissue grown in 96-well high throughput PermaCell plate. A) Plate cover. B) Insert plate with 0.4 µm membrane bottom. C) Reservoir plate. D) H&E stained cross-section of EpiIntestinal™ tissue cultured in the 96-well insert plate. E) Cross-section of insert and bottom plates filled with 100mM KCl showing TEER electrodes.w

Automated TEER For Rapid GIT Screening

Transepithelial Electrical Resistance (TEER) Measurements

Prior to TEER measurements, the EpiIntestinal™ tissues were transferred to sterile 100 mM KCl (150 µL apical, 400 µL basolateral). TEER was measured both manually using an STX HTS EVOM™ electrode in conjunction with an EVOM™ Manual, and automatically using the EVOM™ Auto automated TEER measurement system (World Precision Instruments, LLC, Sarasota, Florida). A schematic of the Permacell™ 96-well system showing the location of the EVOM™ Auto 96 HTS electrodes is shown in Fig. 1E. A comparison of the EVOM™ Manual with the STX HTS EVOM™ electrode and the EVOM™ Auto systems is shown in Fig. 2A-B. Automated readings were made with a read time of 2 seconds per well. Prior to TEER calculation, tissue sample resistance measurements were background subtracted using the resistance values of blank 100mM KCL buffer in the receiver plate of the 96-transwell assembly.

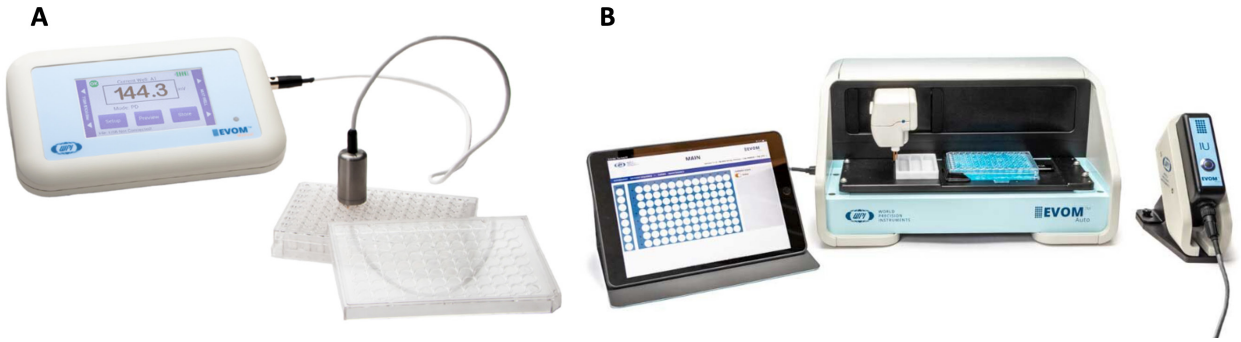


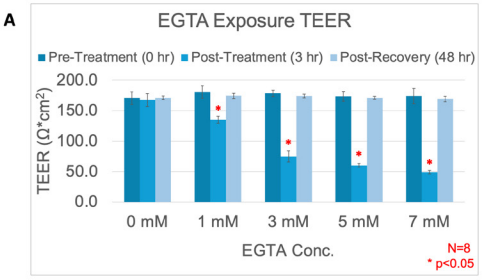
Fig. 2—The EVOM™ Auto allows for high-throughput TEER measurements. A) The EVOM™ Manual w/ STX HTS electrode in a 96-well Transwell plate takes 24 minutes and 3 seconds to measure 96 samples. B) The EVOM™ Auto system for automated measurement of TEER in 96 well plate requires only 4 minutes and 10 seconds to read 96 samples.

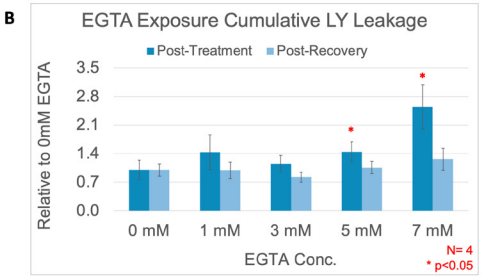
Test Article Addition (Dosing)

Prior to exposure to the test articles, baseline measurements of tissue barrier function (TEER) were taken according to to the methods stated above. Short-term (three-hour) exposure to ethylene glycol tetra-acetic acid (EGTA) was conducted using 1X Hank's Buffered Salt Solution (HBSS) containing 10mM HEPES as a vehicle applied to the apical surface. Long term (6-day) exposure to dextran sulfate sodium salt (DSS) was conducted by adding DSS to the culture medium in both the apical and basolateral compartments. Reversible tissue damage assessed by TEER is expressed relative to the respective vehicle control tissues.

Immunohistochemistry (IHC) Staining

Tissue samples were fixed for 3 hours in 10% formalin, permeabilized for 30 minutes with 1X Tris-Buffered Saline (TBS) containing 0.1% Triton-X-100 and blocked for 2 hours in 1X TBS containing 10% normal goat serum. Monoclonal recombinant Rabbit anti-Claudin 1 antibody (Abcam cat# ab211737, Cambridge, MA) was diluted 1:1,000 in TBS and incubated at room temp for 2 hours with gentle agitation. Goat anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor™ 555 (ThermoFisher cat# A21429, Waltham, MA) was diluted 1:400 in TBS and incubated at room temp for 1 hour with gentle agitation. Nuclei were counterstained with 4',6-diamindino-2-phenylindole (DAPI). Images were acquired using an Olympus FV1000 confocal microscope using both a 2X objective and a 40X oil immersion objective. Representative images for IHC staining is shown in Fig. 3D and Fig. 4D for the EGTA and DSS exposures, respectively.





C

P app (x10⁻⁶ cm/s)					
	EGTA Concentration				
Timepoint	0 mM	1 mM	3 mM	5 mM	7mM
Post-Treatment	6.19	9.16	7.14	9.00	16.59
Post-Recovery	3.39	3.44	2.76	3.60	4.56

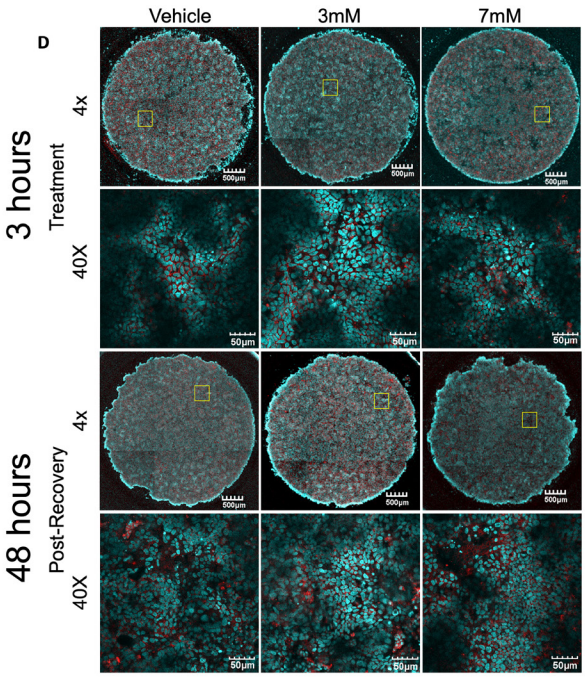
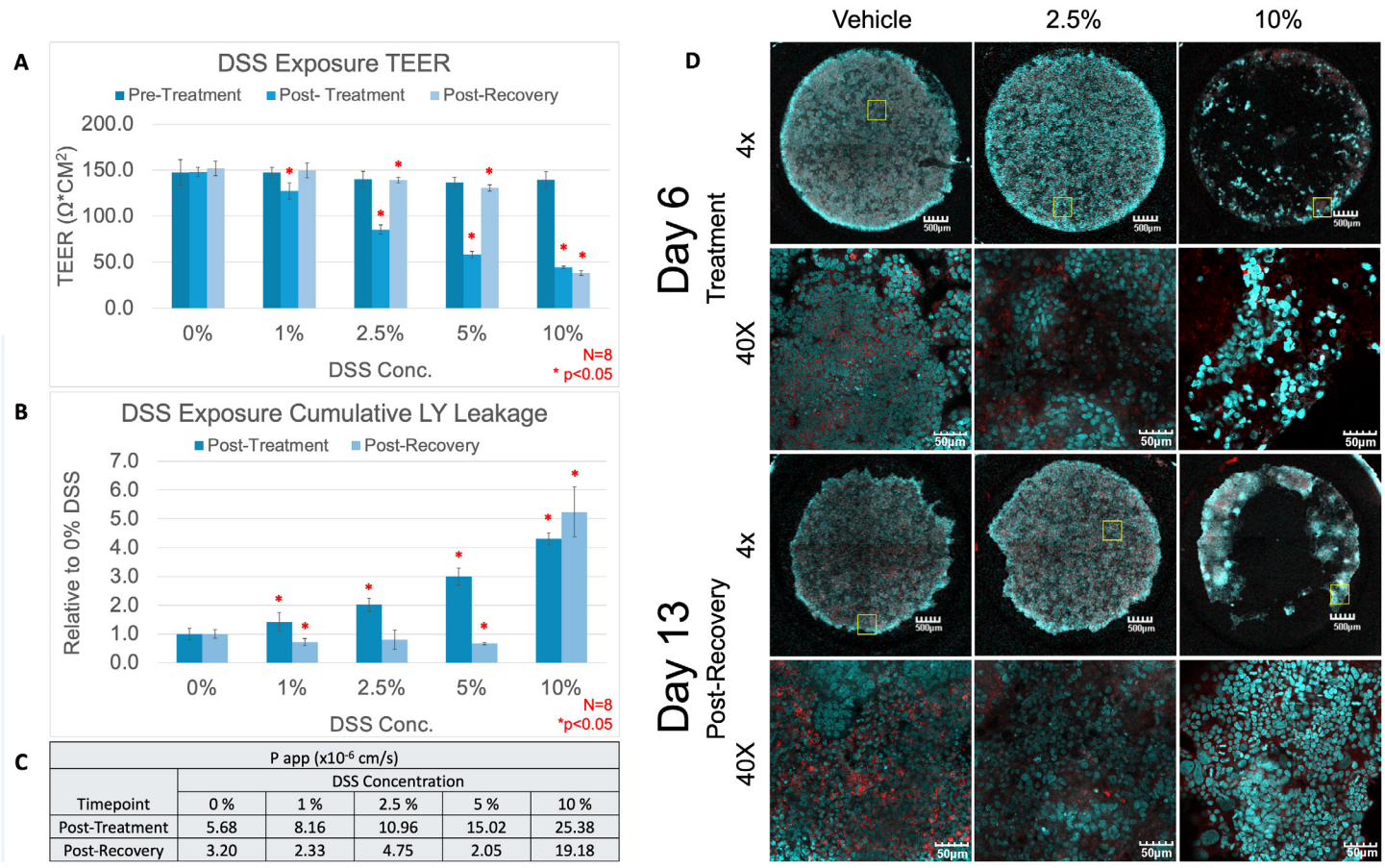


Fig. 3— The EpiIntestinal™ tissue model demonstrates full recovery following 30 hour exposure to EGTA and 48-hour post-exposure incubation. A) TEER measurements. B) Lucifer Yellow (LY) permeability. C) Apparent permeability coefficient for LY. Equation for calculation:

$$P\ app = \frac{\Delta Q}{\Delta t} x \frac{Vr}{A*C}$$

, where ΔQ/Δt is the concentration of LY present in the basolateral compartment following the time elapsed, Vr is the volume of receiver buffer in the basolateral compartment, A is the surface area of the tissue and C is the initial LY concentration applied to the apical surface of the tissue. D) Immunostaining for barrier protein, Claudin 1 (red), counter stained with DAPI (cyan). Error bars = standard dev.



P app (x10⁻⁶ cm/s)					
	DSS Concentration				
Timepoint	0 %	1 %	2.5 %	5 %	10 %
Post-Treatment	5.68	8.16	10.96	15.02	25.38
Post-Recovery	3.20	2.33	4.75	2.05	19.18

Fig. 4—The EpiIntestinal™ tissue model shows prolonged impairment of barrier integrity following a 6-day exposure to 10% DSS and a 7-day (Day 13) post-exposure incubation. A) TEER measurements. B) Lucifer Yellow (LY) permeability. C) Apparent permeability coefficient for LY.

Equation for calculation: $P\ app = \frac{\Delta Q}{\Delta t} x \frac{Vr}{A * C}$ *, where ΔQ/Δt is the concentration of LY present in the basolateral compartment following the time elapsed, Vr is the volume of receiver buffer in the basolateral compartment, A is the surface area of the tissue and C is the initial LY concentration applied to the apical surface of the tissue. D) Immunostaining for barrier protein, Claudin 1 (red), counter stained with DAPI (cyan). Error bars = standard dev.*

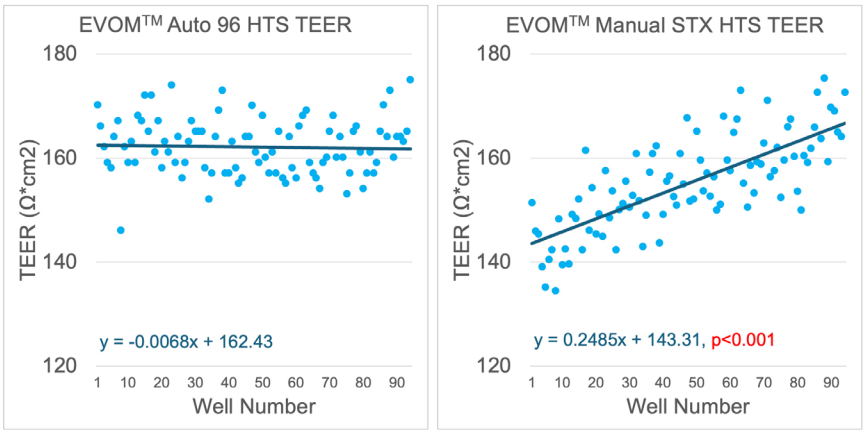
Lucifer Yellow (LY) Permeability

Tissues were transferred to 250µL of 1X HBSS containing 10mM HEPES and 1.98g/L glucose, and 50µL of 100µM LY dye in the same buffer was applied to the apical surface. The basolateral receiver solution (RS) was replaced every 30 minutes for 2 hours. 100µL from each RS sample was used to determine the LY concentration in the RS at each time point. The RS samples were read using a Spectramax M2 plate reader (Molecular Devices, San Jose, CA) at an excitation bandwidth of 445-455nm, an emission bandwidth of 518-538 nm, and a sensitivity setting of 145.

A

EVOM™ Auto 96 HTS			EVOM™ Manual STX HTS		
Avg. TEER (Ω*cm²)	STDEV (Ω*cm²)	Time to read plate	Avg. TEER (Ω*cm²)	STDEV (Ω*cm²)	Time to read plate
162.1	5.4	4m10s	155.1	9.0	24m3s

B



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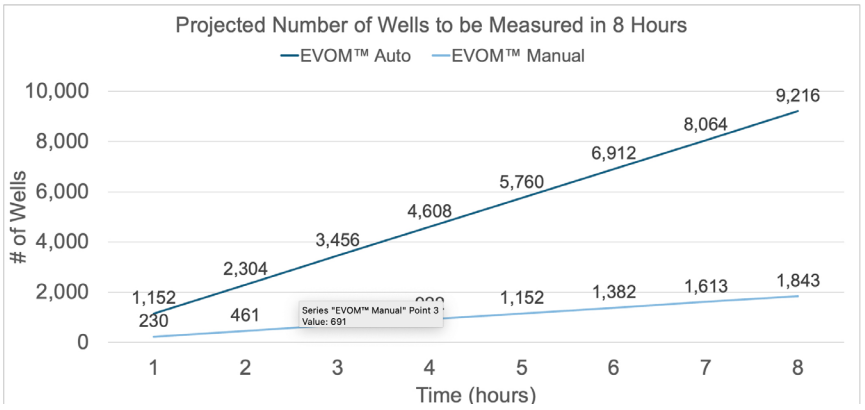

Fig. 5—TEER measurements of EpiIntestinal™ tissues cultured in 96-well PermaCell plate are more consistent using EVOM™ Auto vs EVOM™ Manual measurement systems and are able to be collected more rapidly for ease of use. A) EVOM™ Auto readings were more reproducible and the time to measure the plate was dramatically decreased vs. the EVOM™ Manual. B) TEER values from the EVOM™ Manual were less stable during the measurement period than the EVOM™ Auto increased moderately during the manual measurements (p<0.001). C) Projected number of wells that can be read in eight hours based on time to read a single plate.

Conclusions

- TEER is a non-destructive analytical technique for functional readout of barrier integrity which can be used to monitor changes to 2D monolayers and 3D tissues models and correlate *in vitro* and *in vivo* phenomena.
- TEER measurements of the EpiIntestinal™ organotypic tissue model can be used to predict the GIT profile of therapeutics, and more specifically oncology drug-induced diarrhea, a common side effect of oncology drugs which can limit drug utilization.
- This study shows the utility of the automated TEER measurements to screen therapeutics on 96-well plates. EVOM™ Auto is an instrument that overcomes the challenges of manual TEER measurements and bolsters the ability to utilize TEER for high-throughput screening.
- The use of EGTA and DSS on the EpiIntestinal™ tissue model, can be used to alter barrier function and evaluate a dose dependent response by TEER measurement, Claudin-1 IHC, and Lucifer yellow permeability assay. Compromised barrier function can be detected by TEER measurement and confirmed by LY permeability assay and immunostaining. The withdrawal of the EGTA and DSS treatments recovers the barrier integrity, at least partially, as verified by TEER measurement and LY permeability assay.
- We anticipate that the EpiIntestinal™ model together with the automated TEER measurement capabilities of the EVOM™ Auto will be very useful to model normal GI physiology, GI pathology and to screen the potential toxic effects of drug candidates in pharmaceutical development programs.

References

1. *Peters, et al., Toxicological Sciences, 2019 Mar 1;168(1):3-17*