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Protocol

Small Intestine Tissue Model (SMI-100 and SMI-200-FT) Measurement of Transepithelial Electrical Resistance (TEER) and Potential Difference (PD)

I. Purpose

Tissues such as the small intestinal epithelium produce tight junctions between cells which inhibit the permeation of low molecular weight solutes across the tissue. The formation of tight junctions also inhibits the ability of electric current to flow across tissue, conferring an electrical resistance property to these tissues. Measurement of transepithelial electrical resistance (TEER) provides a convenient indicator of tight junction development and barrier function. TEER of excised small intestine epithelial tissue has been reported to be 12-120 Ω * cm^2 (**1**, **2**). Small intestine epithelial tissues are also electrogenic - they form and maintain a transepithelial electrical potential difference (PD). The PD is also an indicator of the barrier function and viability of the tissue.

This protocol describes measurement of TEER and PD of EpiIntestinal in vitro tissues using a convenient commercially available epithelial voltohmmeter. The instrument operates by means of an alternating current which is non-destructive to the tissue. TEER and PD measurements are useful for quality control as well as determining the effect of test agent effects on intestinal barrier integrity for quality control, toxicology, and basic research applications.

II. Required Materials

EVOM™ Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL)
- Endohm™ Tissue Resistance Measurement Chamber (World Precision Instruments, Sarasota, FL)
- Endohm-12: used for EpiIntestinal-100 (SMI-100 or SMI-200-FT) tissues

Potassium chloride (KCl, 100 mM)
Forceps

III. Procedure

Note: Consult the EVOM™ Epithelial Voltohmmeter and Endohm™ Tissue Resistance Measurement Chamber manuals for additional information and instructions.

a) If culturing of tissues is to be continued after measurement, Endohm™ Tissue Resistance Measurement Chamber should be sterilized (see Endohm™ Tissue Resistance Measurement Chamber manual). All calibration and measurement operations should be performed in a tissue culture hood utilizing sterile technique and sterile buffers.

b) Connect the Endohm™ Tissue Resistance Measurement Chamber to the instrument with the electrode leads (longer electrode lead wire should attach to the bottom portion of the chamber to obtain correct polarity). If electrodes have been stored dry, fill the chamber with enough KCl (100 mM) to immerse the top electrode. Equilibrate the electrodes for about 20 minutes with the power off prior to checking the calibration. To check the calibration, turn the Mode knob to V and turn the power on. Adjust the potentiometer with a screwdriver to

obtain a zero reading, if necessary. Next, turn the Mode knob to R (resistance). Adjust the potentiometer with a screwdriver to obtain a zero reading if necessary.

c) With a small amount of KCl in the Endohm™ Tissue Resistance Measurement Chamber (1.5 mL for Endohm 12), place a blank insert (For SMI-100 or SMI-200-FT, use MILCEL-MTK) into the chamber. Add enough KCl to the top surface of the insert to completely cover the membrane surface to a depth of 4-5 mm and adjust the top electrode so that it is close to, but not making contact with, the top surface of the insert membrane. Background resistance of the blank insert should be about 5-20 Ω. cm^2. The instrument is now ready for tissue measurements.

d) Dispense a small volume of room temperature KCl into the well(s) of a standard 6-well tissue culture plate. Transfer the inserts to be measured to the 6-well plate and gently rinse the top surface of tissues by adding 0.5 mL of KCl and aspirating. Add enough KCl to the top of each insert to completely cover the surface of the tissue and submerge the top electrode (4-5 mm). Transfer the individual tissues into Endohm™ Tissue Resistance Measurement Chamber, and replace top electrode (make certain that the electrode does not touch the tissue). With the Mode knob set to R, push the Measure button and record the resistance. Change the Mode knob to V and record the PD reading.

e) Calculate TEER value: Multiply the resistance measurement from the Endohm device by the area of the tissue. The surface area for the EpiIntestinal tissue models (SMI-100 and SMI-200-FT) = 0.6 cm^2.

f) Decant KCl from the top surface of the tissue insert and return the tissue to the culture vessel for longer term studies. Alternatively, process the tissue for histology, transmission electron microscopy, RNA isolation, etc.

References:

(1) Gupta V, Doshi N, and Mitragotri S (2013) Permeation of insulin, calcitonin, and exenatide across Caco-2 monolayers: measurement using rapid 3-day system. PLOS ONE 8: e77136.

(**2**) Artursson P, Ungell AL, Löfroth JE. Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. Pharm Res. 1993 Aug;10(8):1123-9.