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Transepithelial/endothelial Electrical Resistance (TEER) theory and applications for microfluidic body-on-a-chip devices

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ABSTRACT

Transepithelial/endothelial electrical resistance (TEER) is a valuable method for assaying in vitro barrier tissue integrity, and is becoming an important measurement for body-on-a-chip barrier tissue devices due to its usefulness and non-invasive nature. The measurement concept is relatively straightforward, with TEER measurements performed by applying an AC electrical signal across electrodes placed on both sides of a cellular monolayer and measuring voltage and current to calculate the electrical resistance of the barrier. However, details of the setup, measurement circuit, and applied electrical signal must be properly designed for accurate measurements. Several main factors contribute to errors and variability in the measurement of TEER values, and while many of these factors can be reasonably controlled with little effort in Transwell®-type culture conditions, these factors can become major issues in body-on-a-chip devices without proper design. This mini-review outlines several important aspects of TEER measurements, including the basic theory, commercial systems used to perform measurements, major factors that contribute to measurement errors, and the application of TEER measurements to current body-on-a-chip barrier tissue devices, with the aim of providing guidance for the design of novel body-on-a-chip systems.

Introduction

Numerous *in vitro* models have been developed to study molecular transport across barrier tissues composed primarily of epithelial and endothelial layers[1]. These epithelial and endothelial cell layers serve important and varying roles throughout the human body, providing a barrier between different tissues while also selectively transporting molecules across the barrier. These cell layers are characterized by the presence of tight junctions (*zonula occludens*), which are able to specifically regulate the flow of ions, solutes, and cells through the paracellular space[2], and adherens junctions, which regulate cell-cell interactions[3].

Transport across these barrier tissues is a function of both active and passive transport[4]. Due to the pharmacological significance of drug transport through epithelial and endothelial cell layers, it is imperative to develop methods to quantify barrier tissue function. In order to properly study the function of a barrier tissue, especially an *in vitro* cultured barrier tissue, the cell layer must have high integrity with established tight junctions connecting adjacent cells to produce passive transport similar to that found *in vivo*. Transepithelial/endothelial electrical resistance (TEER) is a rapid, non-invasive method for quantifying barrier tissue integrity by measuring the electrical resistance across the tissue. This mini-

review aims to discuss the theory behind TEER, describe the proper implementation of TEER, and highlight the application of TEER to body-on-a-chip or human-on-a-chip systems incorporating various barrier tissue models.

Methods for Measuring TEER

(1) *Resistance-based measurements*

TEER measurements are generally performed by placing electrodes on both sides of a cell layer grown on a semipermeable membrane, applying an AC signal, and measuring both current and voltage across the cell layer. TEER can also be measured on cells grown on a surface containing multiple electrodes with a proper configuration. In most TEER measurements a standardized configuration is used incorporating electrodes of silver and silver/silver chloride (Ag/AgCl) and using a signal frequency of 12.5 Hz. A low current signal of approximately 10 μA is applied to the cell layer through the silver electrodes, and Ag/AgCl electrodes on either side of the membrane measure the voltage. With this configuration, the calculation of TEER becomes simple, using Ohm's Law to determine resistance from the current and voltage passing through the cell layer. The simplified model of the resulting electrical circuit for the TEER measurement contains electrical resistances for both the transcellular and paracellular pathways in parallel (Figure 1). The equivalent resistance (R_{total}), combining both current paths, is given by the combination of the resistance through the transcellular pathway (R_{tc}) and the paracellular pathway (R_{pc}) as:

$$\frac{1}{R_{total}} = \frac{1}{R_{tc}} + \frac{1}{R_{pc}}$$

Due to the high resistance of cell membranes, the current predominantly flows through the paracellular route, making R_{total} approximately equal to R_{tc} as the conductance through the membrane is sufficiently small and can be neglected for many applications. Additional factors, such as medium resistance, electrode-medium interfacial resistance, and resistance of the semipermeable membrane all contribute to the total resistance. While strategies to reduce the contribution of these variables will be discussed later, a simple approach is to subtract from all resistance measurements the resistance of an identical testing configuration without the cell layer (a blank), approximating the resistance of the cellular layer:

$$R_{cells} = R_{total} - R_{blank}$$

The measured electrical resistance is inversely proportional to the area of the cell layer, since an increased area provides more effective parallel paths for current to pass from one side to the other. In order to allow for TEER measurement comparisons among different experimental setups, the electrical resistance is normalized to the area by multiplying the resistance by the membrane area:

$$TEER\ (\Omega * cm^2) = R_{cells}\ (\Omega) \times Area\ (cm^2)$$

TEER measurements using this simplified electrical circuit can be performed using one of several commercially available systems, such as the EVOM2 (World Precision Instruments, Sarasota, Fl) or the Millicell ERS (EMD Millipore, Darmstadt, Germany). These systems use a 12.5 Hz square-wave AC signal to measure TEER, and are primarily designed for use with Ag/AgCl "chopstick" electrodes in Transwell® permeable cell culture supports or similar culture devices. However, both the EVOM2 and Millicell ERS can be adapted for use with custom silver and Ag/AgCl electrodes[5].

(2) *Impedance-based measurements*

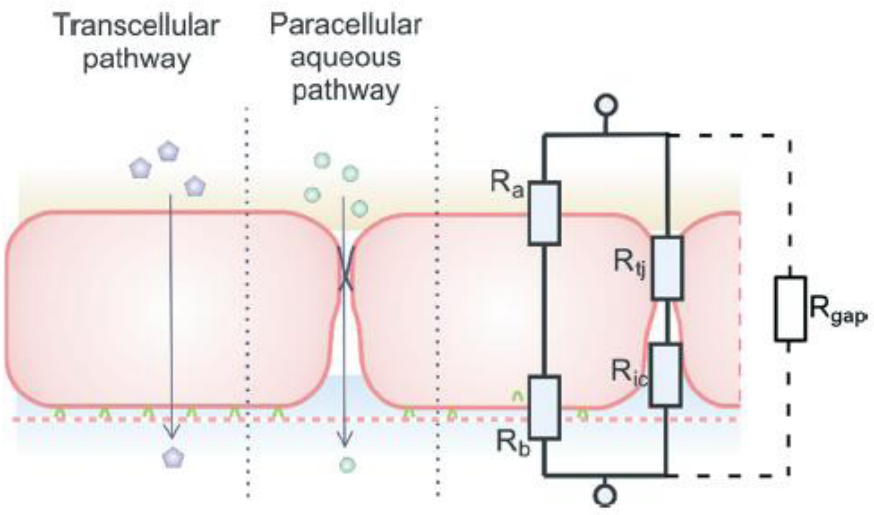

Figure 1. Equivalent circuit model of a cellular monolayer consisting of a transcellular route (R_a + R_b) and paracellular route (R_{tj}+R_{ic}) for current passing through the membrane. Reproduced from Odijk et al[7] with permission of the Royal Society of Chemistry.

A more advanced technique, known as impedance spectroscopy, can be used to determine TEER in a more robust manner and requires fewer assumptions regarding the electrical behavior of the system. Impedance is the complex ratio of voltage to current, and includes contributions from resistive, capacitive, and inductive components of the circuit. When using impedance spectroscopy to measure TEER, measurements are taken across a range of frequencies, and the resulting impedance is plotted as a function of frequency. Since capacitive reactance (the effective resistance provided by a capacitor) decreases as frequency increases while resistance is frequency independent, the impedance spectrum can show the contributions of specific capacitances to the overall impedance. Figure 2 shows the typical impedance spectrum for a TEER system and the equivalent circuit diagram. For the commercial TEER systems that use silver and Ag/AgCl electrodes, the 12.5 Hz frequency is high enough to reduce the contribution from the electrode-medium interface, but low enough to keep the capacitive reactance of the cell membrane high and force the current through the paracellular route. While the frequency is high enough to reduce capacitive impedance, the resistance measured will still contain some capacitive impedance contributions from the experimental setup which can be partially ameliorated by subtracting the measured resistance of a reference setup. To perform impedance spectroscopy, a more complex measurement setup is required which calls for more advanced equipment such as a lock-in amplifier and function generator[6]. The ECIS (Applied BioPhysics, Troy NY), which utilizes arrays of electrodes on a single surface, and cellZscope® (nanoAnalytics, Munster Germany) are both commercially available devices which provide impedance spectroscopy measurements. Impedance spectroscopy provides multiple advantages over non-impedance-based methods for measuring TEER: it removes the influence of the electrode-medium interfacial resistance on impedance calculations and it enables calculation of the capacitance of the cell membrane. Additionally, it can be used as a system optimization procedure to determine the optimal AC frequency for single frequency TEER measurements in any given system.

Factors Influencing TEER Measurements

TEER measurements, while very powerful, are also subject to variability. Many factors contribute to the measured TEER value and must be controlled in order to obtain accurate and reliable results. In addition to the cellular monolayer, the medium, the supporting material for the cell layer (typically a semi-permeable membrane), and the electrode/medium interface all contribute to the final impedance measurement. Subtracting the resistance measurement of a blank removes much of the variability. However, this method is prone to error in custom microfluidic devices, since chip-specific conditions such as electrode placement and temperature may vary between the blank system and tissue-containing systems. One alternative to this subtraction method utilizes two current-passing electrodes on each side of the membrane. By subtracting resistance measurements of current paths between electrodes on the same side of the membrane, the contribution to total resistance from components such as the medium and electrodes/medium interface can be removed[6]. The layout of this device, with the incorporation of multiple electrodes, is shown in Figure 3.

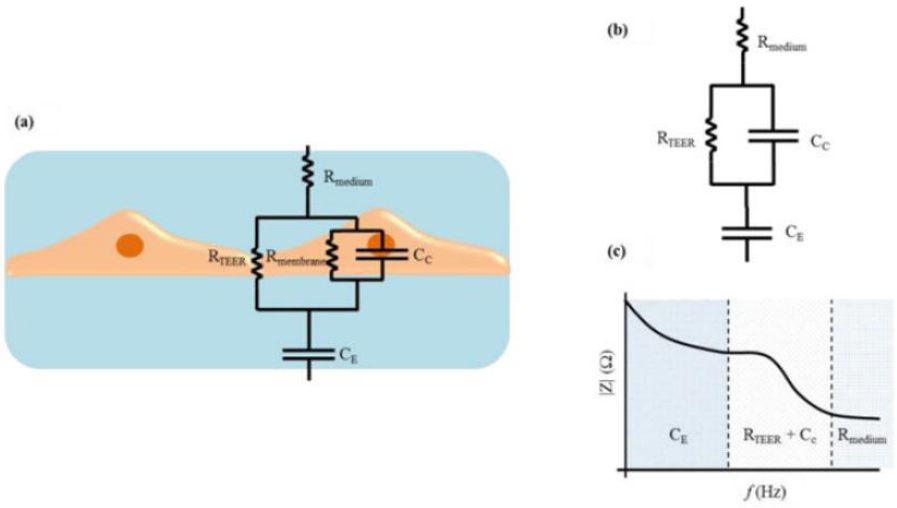

Figure 2. Circuit schematic of major contributors to impedance of the cell layer. (A) Impedance-based equivalent circuit diagram of a cellular monolayer that includes capacitance of the cell membrane (C_c), resistance of the cell membrane ($R_{membrane}$), resistance of the paracellular route (R_{TEER}), resistance of the medium (R_{medium}), and electrode capacitance (C_E). This circuit can be simplified to (B) due to high membrane $R_{membrane}$ resistance forcing current through the other paths. (C) Typical impedance spectrum for barrier tissue cultures from circuit in (B). From Srinivasan et al 2015, *Therapeutic Innovation & Regulatory Science* with permission[24].

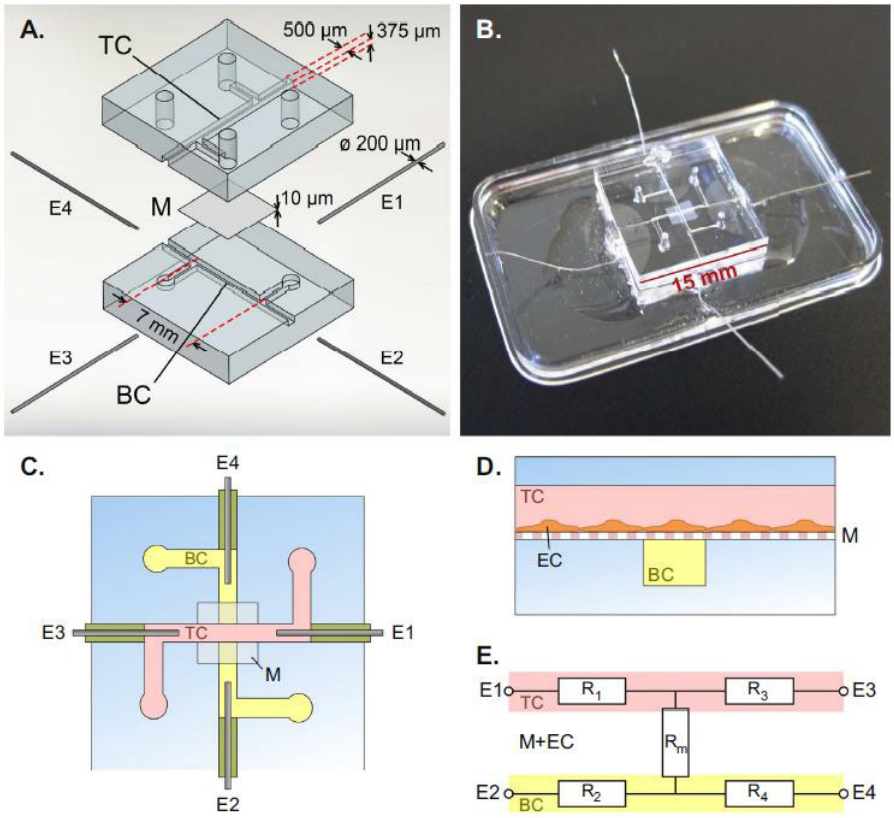

Figure 3. Device incorporating multi-probe method with on-device subtraction. Layout of the microfluidic device with two electrodes on each side of a cell-containing membrane (A), image of an assembled device with top channel (TC), bottom channel (BC), membrane (M), endothelial cells (EC), and electrodes 1 through 4 (E1-E4) (B), schematic of probes (C), cross-sectional schematic of cell-covered membrane (D), and equivalent circuit schematic for the system, allowing for on-device subtraction (E). Reprinted from van der Helm et al 2016[6] with permission from Elsevier.

The current distribution through the cell layer plays a role in TEER measurement accuracy and is affected by membrane area, probe design/shape, and microfluidic channel geometry[7]. For many TEER measurement systems, the current distribution across the membrane is non-uniform, thus reducing the effective area of the membrane and resulting in an overestimation of the resistance of the cellular barrier. The error due to current non-uniformity can be reduced by designing electrodes with a large surface area relative to the cellular monolayer[5].

Probe configuration is also an important consideration in a TEER system. A two point probe method consists of a single electrode on each side of the tissue, each of which is a current-passing electrode and a voltage measurement electrode. A four point probe method separates the voltage measurement and current-passing functions into separate electrodes on each side of the cell layer. By separating the current-passing and voltage measuring electrodes, the four point probe method has the advantage of eliminating the contribution of contact and lead resistances[8]. While lead resistance is typically negligible relative to the measured resistance in TEER systems, the contact resistance due to the electrode-medium interface can be significant, particularly when using electrodes made of inert materials such as platinum[9].

Body-on-a-Chip Applications

Body-on-a-chip systems are cell-based microfluidic devices that aim to recapitulate certain aspects of *in vivo* structure and/or function in order to study disease models, drug efficacy and toxicity. These systems have defined microenvironments to mimic *in vivo* conditions of the cells and often include microfabricated chips to provide a functional measurement of cell performance[10,11]. Due to the role of barrier tissues in regulating molecular transport and homeostasis throughout the body, barrier tissue models are playing an increasingly important role in developing body-on-a-chip systems, particularly in drug discovery[12]. This section will review the integration of barrier tissue models and the application of TEER in microfluidic body-on-a-chip devices.

The blood-brain barrier (BBB) is a selective barrier for the central nervous system that regulates the transport of nutrients and oxygen into the brain. The BBB consists of an endothelial cell layer supported by astrocytes, pericytes, neurons, and microglia, together forming the neurovascular

unit. The BBB is a highly impermeable barrier, and also possesses hydrolytic enzymes to break down exogenous compounds[13]. Therefore, BBB models are critical in developing drugs that can permeate the BBB and affect the brain. Multiple *in vitro* models of the BBB have been developed[5,14-17], primarily consisting of endothelial cells and astrocytes seeded on opposite sides of a permeable membrane. In the blood-brain barrier system shown in Figure 4, monitoring of TEER enabled optimization of culture conditions and allowed for drug transport testing in the device[15]. In this particular device, TEER was measured using a four point probe method with custom Ag/AgCl electrodes that were connected to the Millicell ERS meter. Overall, these BBB models have been successful in demonstrating high TEER values characteristic of a functional BBB as well as selective permeability and responsiveness to drug dosage.

The gastrointestinal (GI) tract epithelium controls the uptake of nutrients from the GI tract into the bloodstream. Thus, models of the GI tract can be an important tool in studying the pharmacokinetics of orally administered drugs. Shuler *et al.* in 2016 developed a multi-organ chip containing GI tract epithelium utilizing a pump-less, unidirectional flow system to achieve physiologically relevant shear stress[18]. In this device, the GI tract epithelium was created by seeding Caco-2 cells on a porous membrane, and TEER measurements were performed using a four-probe method with silver tubes containing Ag/AgCl electrodes on each side of the membrane. The tubular structure of the current-passing silver electrodes created

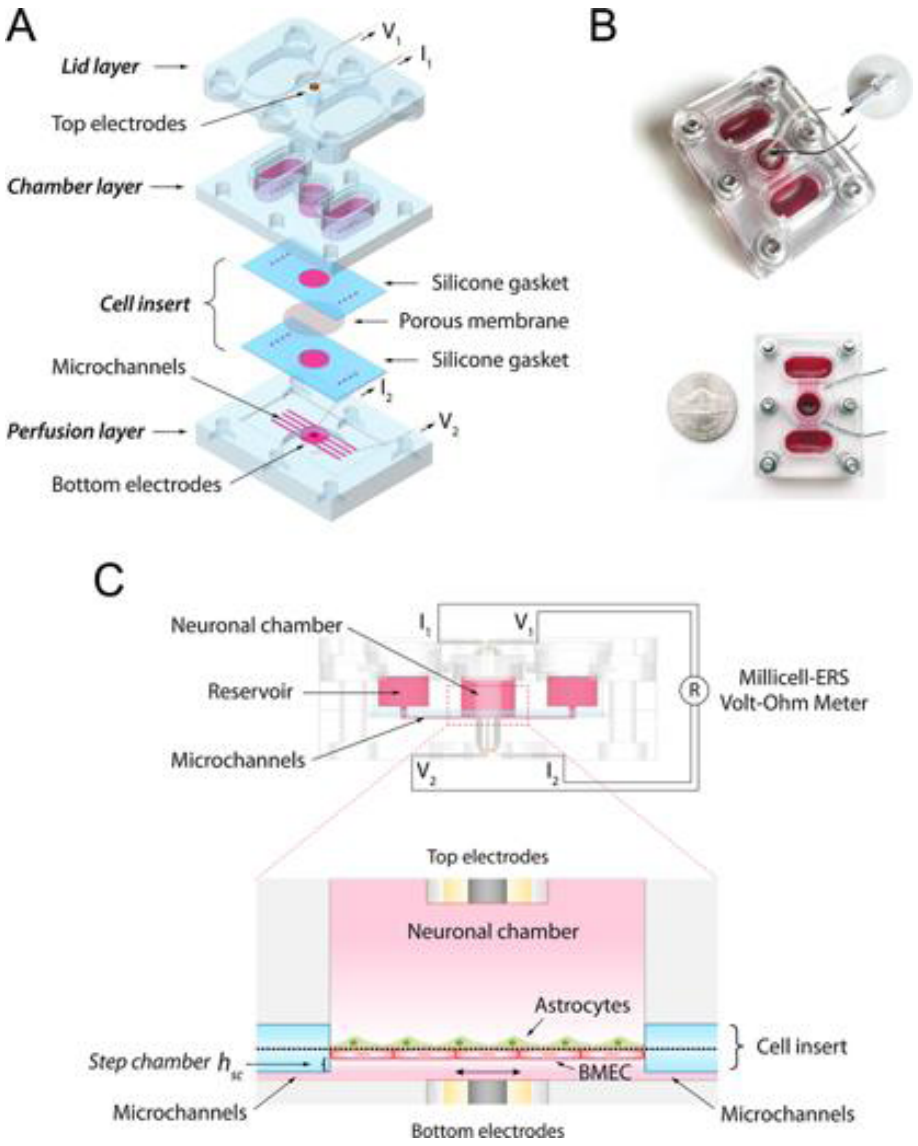

Figure 4. Blood-brain barrier body-on-a-chip device with integrated four point probe TEER measurement. Schematic layout of the multi-layer device (A), images of the assembled system (B), and schematic of cell layout and TEER configuration inside the system (C). From Wang et al. 2016[1515], © 2016 Wiley Periodicals, Inc., used with permission.

a more even current distribution across the membrane, allowing for more accurate TEER measurements.

While the blood-brain barrier and GI tract are two of the most prevalent barrier tissue models, several others have been studied such as skin, lung, kidney, and ocular barrier models[19-22]. More recently, TEER has been applied to a blood-tumor barrier, which has exciting implications for cancer drug delivery[23]. In all of these barrier models, the use of TEER measurements is critical for establishing and measuring the integrity of the barrier function, to enable studies on both active and passive transport properties as well as drug effects on the barrier itself.

Conclusions

In vitro barrier tissue models are becoming increasingly important as tools for drug discovery and for studying pharmacokinetics. As body-on-a-chip systems continue to improve and become more complex, barrier tissues for drug and nutrient uptake, transport within the system, and elimination will need to be incorporated to better model human physiological response to drug compounds and organ-organ interactions. TEER measurement will be a vital technique for the characterization of barrier integrity in these systems. While many TEER measurement configurations are possible, those that provide the most reliable and robust TEER values employ a 4-probe method, an optimal AC frequency signal, and electrodes designed to provide a uniform current distribution. While four-probe methods offer advantages, two-probe methods can also provide accurate TEER measurements, particularly when multiple current paths are used to obtain an average resistance value, for instance by using many two-point pairs. Reliable TEER values can be obtained without using impedance spectroscopy, although impedance spectroscopy provides additional information about TEER circuit capacitance and is ideal for determining an optimum measurement frequency. Due to the simplicity of the method, its ability to provide long-term, non-invasive data, and the existence of commercially available systems, TEER is ideal for integration into body-on-a-chip systems. The configurations and considerations outlined here should provide a guide for system design as well as address many issues that may arise in incorporating TEER measurements into increasingly complex microfluidic body-on-a-chip devices due to unique design constraints for each unique system.

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Declaration of Conflicting Interest

Hickman (Chief Scientific Officer) is associated with Hesperos, LLC, a start-up company developing multi-organ chip based models of the body.

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