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Advances in TEER measurements of biological barriers in microphysiological systems

Hojjatollah Nazari[a], Jesus Shrestha[a], Vahid Yaghoubi Naei[a], Sajad Razavi Bazaz[a], Milad Sabbagh[a], Jean Paul Thiery[b], Majid Ebrahimi Warkiani[a,c*]

[a] School of Biomedical Engineering, University of Technology Sydney, Sydney 2007, New South Wales, Australia Australia
[b] Guangzhou Laboratory, Bioisland, Guangzhou 510320, China
[c] Institute of Molecular Medicine, Sechenov University, 119991 Moscow, Russia

* Corresponding author at: School of Biomedical Engineering, University Technology Sydney, Sydney, New South Wales 2007, Australia. Email address: majid.warkiani@uts.edu.au (M.E. Warkiani)

Abstract

Biological barriers are multicellular structures that precisely regulate the transport of ions, biomolecules, drugs, cells, and other organisms. Transendothelial/epithelial electrical resistance (TEER) is a label-free method for predicting the properties of biological barriers. Understanding the mechanisms that control TEER significantly enhances our knowledge of the physiopathology of different diseases and aids in the development of new drugs. Measuring TEER values within microphysiological systems called organ-on-a-chip devices that simulate the microenvironment, architecture, and physiology of biological barriers in the body provides valuable insight into the behavior of barriers in response to different drugs and pathogens. These integrated systems should increase the accuracy, reproducibility, sensitivity, resolution, high throughput, speed, cost-effectiveness, and reliable predictability of TEER measurements. Implementing advanced micro and nanoscale manufacturing techniques, surface modification methods, biomaterials, biosensors, electronics, and stem cell biology is necessary for integrating TEER measuring systems with organ-on-chip technology. This review focuses on the applications, advantages, and future perspectives of integrating organ-on-a-chip technology with TEER measurement methods for studying biological barriers. After briefly reviewing the role of TEER in the physiology and pathology of barriers, standard techniques for measuring TEER, including Ohm's law and impedance spectroscopy, and commercially available devices are described. Furthermore, advances in TEER measurement are discussed in multiple barrier-on-a-chip system models representing different organs. Finally, we outline future trends in implementing advanced technologies to design and fabricate nanostructured electrodes, complicated microfluidic chips, and membranes for more advanced and accurate TEER measurements.

Keywords: transendothelial/epithelial electrical resistance, barrier-on-a-chip, microphysiological systems, organ-on-a-chip, microfluidics

1 Introduction:

Transendothelial/epithelial electrical resistance (TEER) is a biophysical property that is characteristic of biological barriers and analyzed to improve our understanding of different diseases (Majima et al. 2017). Studying this property of biological barriers can assist in deepening our knowledge about the disruptions that occur in diseases or during interactions of barriers with various agents such as drugs, nanoparticles, biological cues, and toxins (Elliott and He 2021b; Jia et al. 2020; Xiao et al. 2020). TEER values reflect the electrical characteristics of an epithelial layer that impact biological features, including the status of cell junctions, cell polarity, cell layer thickness, activity of transporters, and state of confluency (Chaing and Tu 2019; Tu et al. 2021). Various protein complexes assemble into intercellular junctions to establish physiological barriers. Early adhesion stages allows the nucleation of E-cadherin-mediated adherent junctions, which serve as a scaffold for the development of an actin-based adhesion belt and desmosomes, followed by tight junctions, which play a leading role by creating leakproof seals through the tight apposition of the plasma membrane of adjacent cells (Otani and Furuse 2020; Wanat 2020). TEER measurements can provide continuous, noninvasive, real-time measurements and a label-free mode of tracking tight junction functionality in cellular monolayers (Bagchi et al. 2019; Sakolish et al. 2016a). Therefore, they provide measures of cell layer selective permeability to ions, nutrients, water, and drugs (Elbakary and Badhan 2020; Sarmento et al. 2012; Srinivasan and Kolli 2019). Furthermore, TEER can provide information about real-time ionic conduction of the intercellular junctions (Muendoerfer et al. 2010; Sarmento et al. 2012).

To evaluate the electrical properties of a cellular sheet using TEER, a pair of extracellular electrodes dispatch current or voltage on either side of the cell barrier to estimate its integrity as a sum of paracellular and transcellular resistance. Commercially, TEER measurement systems for *in vivo* and *in vitro* studies are based on two mechanisms. The first mechanism is based on Ohm's

Law, in which the measurement of resistance comprises a pair of handheld chopstick electrodes in transwell permeable support arrangements employing a single alternating current (AC) square wave for Ohmic measurements (Meter and Manual 2000; Srinivasan et al. 2015a). The second mechanism is based on the concept of impedance spectroscopy, in which the instrument implements a pair of sensing and counter electrodes on either side of a cell monolayer. This technique generates a broad spectrum of frequency AC sweep for the measurement of both the resistance and capacitance properties of the endothelial or epithelial layer (Benson et al. 2013; K'Owino and Sadik 2005; Kalvøy et al. 2009; Kontturi et al. 1993; Pliquett and Prausnitz 2000; Soley et al. 2005; Srinivasan et al. 2015a). Impedance spectroscopy utilizes a low-amplitude AC excitation signal with a frequency sweep and measures the current's amplitude and phase response. The commercial systems are limited by low reproducibility of measurements due to low sensitivity in the proper positioning of the electrodes and lack of uniformity in distributed currents, leading to their infrequent use for *in vitro* studies (Yeste et al. 2016). Furthermore, these systems are restrained to a static arrangement, causing subtle differences in cell morphology and barrier permeability. On the contrary, *in vivo* transport processes are influenced by different factors such as hemodynamic shear stress and the flow rate of bio-fluids (Lee and Leong 2020; Wang et al. 2020b; Wu et al. 2020a; Zhu et al. 2021).

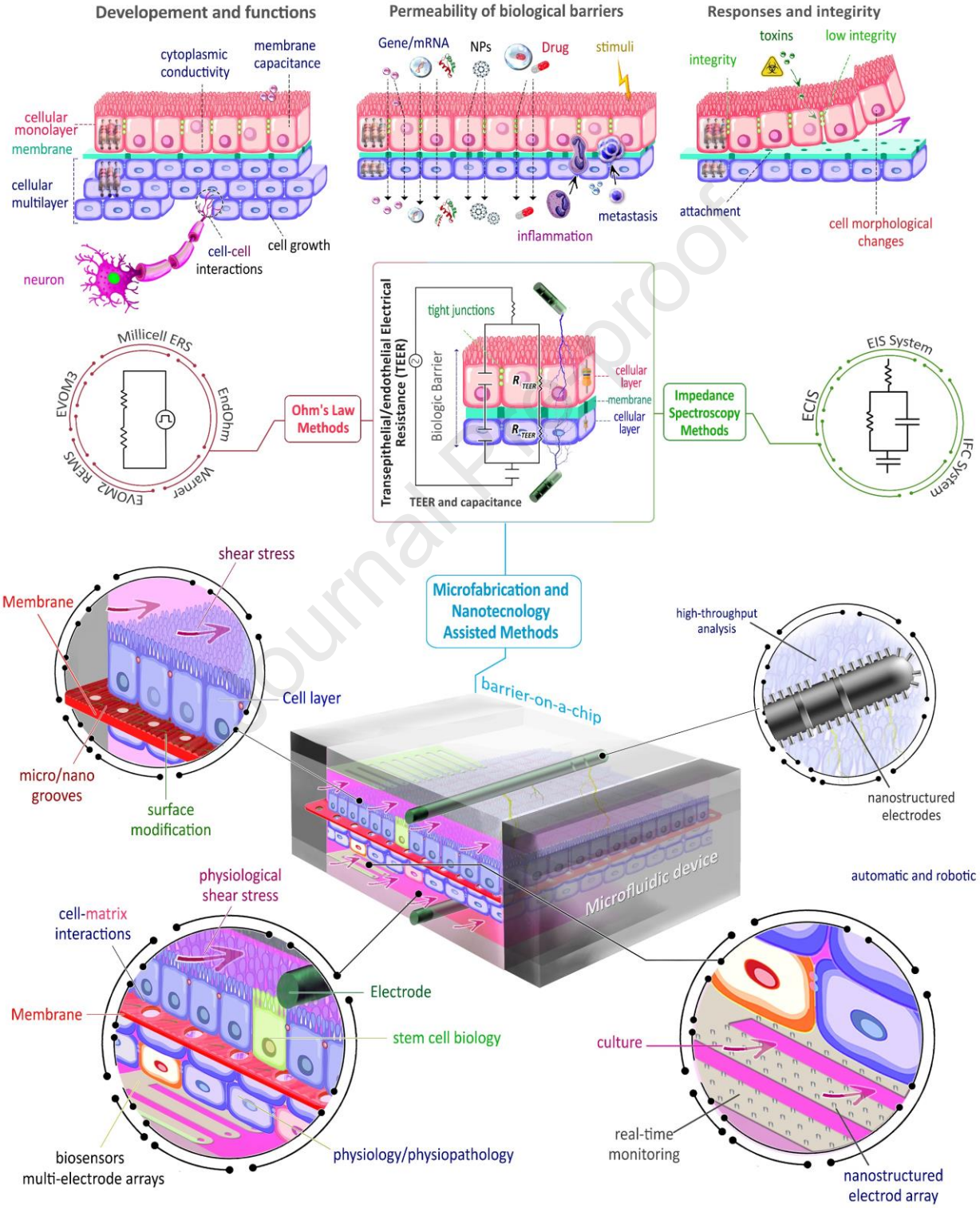
The significant growth of microfluidics, nanotechnology, and stem cell biology has led to the development of a new generation of organ-on-chips, referred to as biological barrier-on-chip. These microfluidic devices can assist in measuring TEER in microenvironments that mimic native tissue-tissue interactions (Liang and Yoon 2021; Miyazaki et al. 2021; Pell et al. 2021). These systems often contain two parallel microchannel cultures separated by a porous membrane and covered with organ-specific cell types (Augustine et al. 2021; Buchroithner et al. 2021; Oddo et al. 2019). Such configurations facilitate perfusion of on-chip grown organ models across a group

of interconnected channels that enhance mimicking physiological cross-talk and circulation across microfluidic culture systems. The barrier-on-a-chip platform incorporates physiological parameters, including biological-fluid-induced shear stress, mechanical strain, ideal gradients regulation, multi-lineage cell layers, and three-dimensional extracellular matrix (ECM) constituents (Cong et al. 2020).

The high capacity and flexibility of barriers-on-chips to be integrated with different sensing systems, including commercial TEER measurement devices or advanced sensors, make them a potential tool for accurate measurement and real-time monitoring of TEER. A variety of microscale sensors and electrodes can be implemented in microfluidic cell culture channels to carry out precise, reliable, and reproducible TEER measurements. The microfabrication methods including patterning and sputtering of metallic elements (gold (Janvier and Kuo 2021; Santbergen et al. 2019; van der Helm et al. 2019a) and indium tin oxide (Asif et al. 2020; Lewis et al. 2018)) on the microfluidic channel substrate and fabrication of microscale electrodes using photolithography and microfabrication techniques have been employed for the integration of TEER measurement systems with biological barriers-on-chips. Therefore, the miniature models of organs can be simultaneously evaluated in TEER measurements, oxygen pressure, and pH (Henry et al. 2017a; Raimondi et al. 2020). Integrating sensing potentials into these systems can further enhance sensitivity and spatio-temporal resolution.

This review presents a comprehensive report on the state-of-the-art advances in TEER measurement in biological barriers-on-chips (organ-on-chip) achieved through nanotechnology, microfabrication, and stem cell biology developments for regenerative medicine and pharmacological applications. We discuss the importance of TEER measurements in the physiopathology of biological barriers and the basics of routine TEER measurement methods. The latest applications of advanced techniques and strategies for increasing the quality of TEER

measurements are then described. Finally, challenges and future trends in developing next-generation TEER securement systems using highly sensitive electrodes, elaborated microfluidic devices, stem cell technology, and membranes will also be discussed (graphical abstract).



Graphical abstract. Applications of TEER measurements for studying development, permeability, and response to biological behavior. The routine TEER measurement, including Ohm's low and impedance spectroscopy-based methods, can be developed using advanced micro and nanofabrication methods. A variety of advantages, including implementing biological shear stress, nanostructured and surface-modified membranes, microelectrodes, cell types, advanced electrodes, biosensors, and arrays, can be implemented in the *in vitro* measuring of TEER inside barriers designed in organ-on-chips.

2 Biological Barriers, the importance of TEER measurements

Biological barriers are essential for the integrity and performance of most organs (Antimisiaris et al. 2021). The development of intercellular connections, especially, tight junctions in epithelial and endothelial cell layers, resulting in a robust barrier between the apical (luminal) and basolateral (abluminal) sides, forming a selectively permeable membrane between compartments, with various biological compositions, regulating transport via the paracellular and intracellular routes (Benson et al. 2013). In general, they regulate the exchange and transportation of different ions, biological agents, exosomes, drugs, nutrients, wastes, and cells regarding the functionality of tissues (figure 1) (Elliott and He 2021a). Furthermore, they protect organs from toxic foreign particles and pathogens and carry out fluid refinement in response to the particular demands of organs to establish homeostasis for physiological functions (Cojocaru et al. 2020; Tsukita et al. 2001; Wanat 2020). As mentioned, TEER measurement is a noninvasive and quantitative method to assess the electrical resistance of biological barrier cellular layers, including endothelial and epithelial monolayers, during *in vitro* and *in vivo* studies. One of the main applications of TEER is to predict the biological membrane integrity in response to different chemical and biological agents such as drugs (Robinson et al. 2018), carbohydrates (Patabendige et al. 2013), hormones, cytokines, viruses, bacteria, extracellular vesicles, and toxins. Transporting these elements across cell membranes relies on different procedures (passive and active), and facilitated transportations, all of which affect the permeability differently (Sarmento et al. 2012; Wegener and Seebach 2014). There are a variety of transport barriers that maintain homeostasis in the whole human body,

including blood–brain barriers, blood–retinal barriers, blood–air barriers, blood–lymph barriers, placental barriers, and gastrointestinal barriers (Chatterjee et al. 2019; Polacheck et al. 2019; Vancamelbeke and Vermeire 2017).

The behavior and capability of biological barriers can change in various diseases due to endothelial or epithelial dysfunctionality. These changes can be investigated by measuring levels of TEER. Therefore, the development of advanced TEER measuring systems, especially for in vitro studies, can assist in the discovery of drugs for the treatment of various diseases. For instance, in oncological studies, the tumor microenvironment can lead to abnormal changes in integrity, quantified using TEER measurement of biological barriers. These changes can lead to phenomena like multidrug resistance, metastasis, or invasion (Fujimoto et al. 2020; Lu et al. ; Salminen et al. 2020). Furthermore, barrier disorders are directly related to other diseases, including neurodegenerative disease, inflammatory disease (such as Crohn's), epilepsy, hypomagnesemia, gastrointestinal tract diseases, or viral infections (Ebrahimi et al. 2020; Liu et al. 2021; Yu et al. 2020). For instance, disruption of the blood-brain barrier, which separates the central nervous system from peripheral blood flow, is one of the main causes of neurodegenerative diseases such as multiple sclerosis (MS), Parkinson's, and Alzheimer's (Griep et al. 2013b; Patabendige et al. 2013).

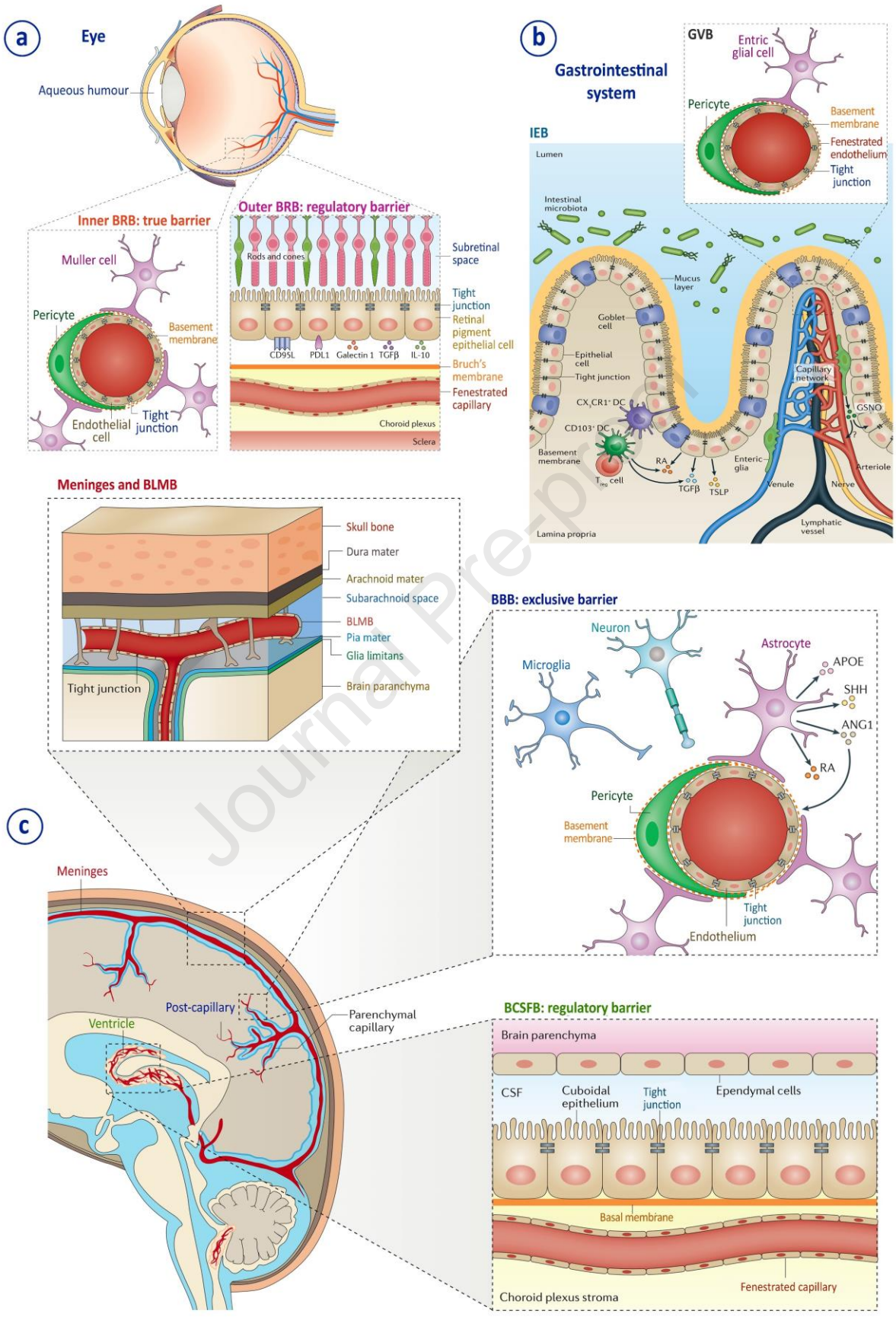


Figure 1. The cellular components of the blood–retinal barrier, intestinal barrier, and blood–brain barriers. Reproduced with permission from (Spadoni et al. 2017).

In the last few years, TEER measurement instruments have been integrated into systems that can model various biological barriers in organ-on-chips. To model human organs as remarkably complex structures, a highly reliable system is required to reconstitute tissue complexity in a chip. Until the last decade, the two primary options used consisted of culturing different human cell lines in vitro (static models) or using animal models, both of which have serious limitations. Although immortalized cells have facilitated in vitro studies, the immortalization process can alter cell behavior as compared with the original cells isolated from tissue or organs. For this reason, primary cells isolated from human samples are preferred, despite the challenges of sustaining their morphological characteristics and metabolic abilities in consecutive passaging.

3 Basics and commercial instruments in TEER Measurement

TEER measurement systems require the establishment of an electrical resistance across a monolayer of cells to determine the tightness of cell–cell connections in the paracellular space (Arık et al. 2018; Douville et al. 2010). TEER measurements are conducted based on two physical principles: Ohm's law and impedance spectroscopy. The basics of TEER measurements and commercial tools in this area are presented and discussed in this section.

3.1 Ohm's Law:

Electrical resistance can characterize the barrier integrity across a cell layer, which can be carried out via Ohm’s law (Srinivasan et al. 2015a). TEER measurements are usually performed by placing an electrode on both sides of a cellular monolayer growing on a semipermeable membrane (Figure 2. a). Then, the electrical resistance (R) can be calculated using Ohm's law as the ratio of the voltage (U) to the current (I) that passes through the cell monolayer. Because direct current (DC) can potentially damage the cells, an AC voltage with a square waveform is a good candidate

to address this challenge (Figure 2. a). Most of the commercially available TEER measurement systems such as Epithelial Volt/Ohm Meter (EVOM2^{TM}, World Precision Instruments, Sarasota, FL) Millicell ERS (EMD Millipore, Darmstadt, Germany) indeed use Ohm's law and apply a 12.5 Hz square wave AC signal (Benson et al. 2013). For the electrodes, these systems use Silver/Silver chloride (Ag/AgCl) "chopstick" electrodes to measure the resistance. The resistance of the cellular layer (R_{Cells}), in units of Ω, can be approximated by:

$$R_{Cells}\ (\Omega) = R_{Total} - R_{Blank},$$

in which R_{Total} is the resistance across the semipermeable membrane and R_{Blank} is the resistance across the semipermeable membrane without a cell monolayer. The measured resistance is inversely proportional to the culture area on the membrane and is calculated as

$$\text{TEER}\ (\Omega cm^2) = R_{Cells}\ (\Omega) \times \text{Area}\ (cm^2)$$

EVOM®

The EVOM® systems from World Precision Instruments (WPI) were the first commercial instruments designed for TEER measurement in tissue and cell culture research. The first generation of this brand is the EVOM2TM, which uses chopstick electrodes (STX2 and STX3) or high throughput screening electrodes (STX100 family). These devices allow users to measure TEER manually without removing the cell culture inserts from the well, making them ideal for use in all laboratories (Meter and Manual 2000). However, because the TEER readings depend highly on the positions of the electrodes, the user must be extremely careful while handling the electrodes and avoid damaging the cell layer. Therefore, electrode positioning reproducibility is a significant concern in this method. Moreover, TEER values can be systematically overestimated due to the variable current density generated by implementing manual STX2 electrodes across the cell layer. The use of cup chambers can address this challenge. For instance, EndOhm chambers provide reproducible resistance measurements of cell monolayers cultured on inserts or culture cups and

are a suitable alternative to handheld STX2/chopstick electrodes. The circular disc electrodes localized above and beneath the membrane create a more uniform current density across the membrane. The newer version of the TEER measurement system, EVOM3TM, provides a better workflow with higher resolution, accuracy, and reproducibility compared to previous designs. REMS AutoSampler is an even more advanced TEER measuring system developed by WPI. It is a PC-controlled system that offers a rapid, precise, and automated TEER measurement in cell culture well plates. The system is easy to operate, reproducible, and decreases the risk of contamination. This system has already been applied to measuring the TEER of in vitro models of some barriers, such as gastrointestinal tract, blood-brain barrier, and pulmonary models, and for drug testing and toxicological studies (World Precision Instruments).

Other Commercial TEER Measurement Systems

The Millicell® ERS Voltohmmeter (Electrical Resistance System, Merck KGaA, Darmstadt, Germany) is similar to the EVOM2^{TM} system and uses handheld electrodes with an Ag/AgCl pellet on the tips to measure voltage (Millicell). An Ussing Chamber (Warner Instruments, Hamden, CT) contains chambers on either side of the membrane with a cell monolayer to allow TEER measurements and data acquisition (Thomson et al. 2019).

3.2 Impedance Spectroscopy

Impedance spectroscopy is a highly reliable electrochemical method to measure the electrical resistance of the cell layer and involves fewer assumptions about the electrical behavior of the system (Benson et al. 2013; Elbrecht et al. 2016b; van der Helm et al. 2019a). Impedance spectroscopy uses a broad spectrum of frequency AC excitation signals to measure the amplitude and phase of the current. The calculated impedance provides additional information about epithelial layer properties, including TEER and capacitance (Xu et al. 2016). The concept of

impedance measurement is illustrated in the schematic in Figure 2b. Electrical impedance (Z) is the ratio of the voltage–time function V(t) and the resulting current–time function I(t):

$$Z = \frac{V(t)}{I(t)} = \frac{V_{\circ} \sin\theta}{I_{\circ} \sin(2\pi ft + \Phi)} = \frac{1}{Y}$$

$$Z = Z_R + {}_jZ_J$$

in which V_o and I_o are the peak voltage and current, f is the frequency, t is the time, Φ is the phase shift between the voltage–time and current–time functions, and Y is the complex conduct admittance. Thus, Z is a complex function and can be described by the modulus |Z| and the phase shift Φ or by the real part ZR and the imaginary part ZI, as illustrated in Figure 2c.

This method has many benefits over non-impedance-based approaches for evaluating TEER. For instance, it eliminates the effect of the interfacial resistance of the electrode medium on impedance estimations and can assess plasma membrane capacitance (Elbrecht et al. 2016b).

Generally, impedance measurements can be categorized into three standard techniques: electrical impedance spectroscopy (EIS), electrical cell-substrate impedance sensing (ECIS®), and impedance flow cytometry (IFC), which are briefly presented in the following section.

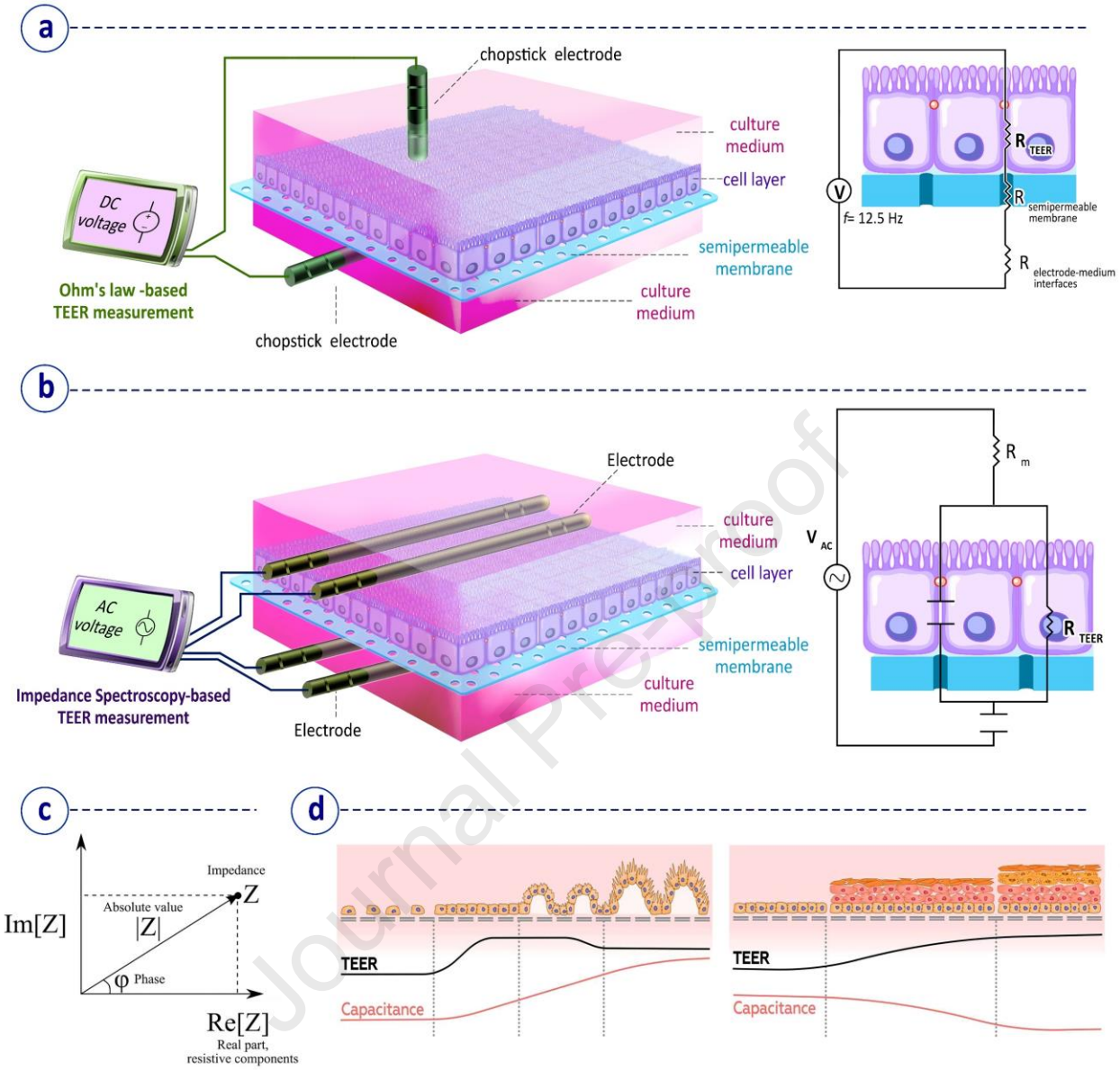


Figure 2. a) The electrical resistance of cells grown on filter inserts was measured using chopstick electrodes. The total electrical resistance includes the Ohmic resistance of the cell layer (RTEER), the cell culture medium in both the upper and lower compartment (RM), the semipermeable membrane of the insert (RI), and the electrode-medium interface (REMI). b) TEER measurement concept based on impedance spectroscopy. c) Components of impedance, phase diagram of complex impedance (illustrates the relationship between Cartesian and polar representations of the complex number. Reproduced with permission from (Gerasimenko et al. 2020). d) Dynamics of TEER and capacitance changes during the growth and differentiation of gut epithelial cells (left) and during the development of reconstructed epidermis (right). Reproduced with permission from (Gerasimenko et al. 2020).

3.2.1 EIS

EIS is an electrochemical method that can be used to measure the potentials of a system that depends on the AC potential frequency. This method can provide detailed information, including cell membrane and cytoplasm characteristics and measure the quantitative changes of cells such as cell types, size, number, and cell growth by sending inducing signals (with different frequencies) and measuring the returning signals (K'Owino and Sadik 2005; Kalvøy et al. 2009; Kontturi et al. 1993; Pliquett and Prausnitz 2000; Soley et al. 2005). Moreover, EIS can provide information regarding the effects of various biomaterials, substrates, and treatments on cell attachment, cell growth, cell toxicity, or inflammation through real-time monitoring (Daza et al. 2013; DePaola et al. 2001; Kozhevnikov et al. 2016; Pérez et al. 2017). In addition, different groups have utilized this method for various tissue engineering applications, including stem-cell research (Hildebrandt et al. 2010; Hildebrandt and Thielecke 2009; Yuste et al. 2018). EIS has also been used as a noninvasive technique for clinical assessments of cancer (breast, skin, and cervical), radiation injury, and ischemic lesions (Aberg et al. 2004; Amini et al. 2018; Brown et al. 2005; Kerner et al. 2002; Strand-Amundsen et al. 2016; Strand-Amundsen et al. 2018). Nevisense™ (Scibase AB, Stockholm, Sweden) is the commercial version of an EIS-based TEER measuring system (Sarac et al. 2020).

3.2.2 ECIS®

ECIS® is another impedance-based system designed by Applied BioPhysics for real-time monitoring of characteristics of cells cultured on solid substrates with a high temporal resolution (Benson et al. 2013). This method is based on applying small AC across the electrode pattern at the bottom of the ECIS®arrays. Cell layers are directly grown on the integrated gold-film electrodes instead of the porous membranes. The close proximity between the cell monolayer and the thin gold electrodes results in high sensitivity measurements. In ECIS®, the impedance of the

cell-covered electrode can be measured by applying a current of chosen frequency or at different frequencies. They provide information regarding phenotypic changes and metabolic activities, the resistance of the cell barriers, and the capacitance of the cell membranes (Amini et al. 2018; Giaever and Keese 1991; Lo et al. 1995).

This technique provides information on cell–cell and cell–matrix interactions, barrier functions, the invasive nature of cancer cells, the monitoring of stem cells, and wound-healing processes (Benson et al. 2013; Nordberg et al. 2017; Rahim and Üren 2011; Szulcek et al. 2014). However, ECIS® has some limitations such as the inability to study transport or transfer phenomena owing to the absence of the basolateral fluid compartment and the inability to monitor cell behaviour inside 3D matrices (Amini et al. 2018).

3.2.3 IFC system

The main benefits of IFC systems is that they can be used to study the electrical behavior of a cell membrane in a single cell. IFC primarily focuses on cells in suspension, providing snapshot information per cell (Cheung et al. 2010). The multiple-frequency-based impedance data obtained from microfluidic IFC facilitates the characterization of different cell sizes, membrane capacitance, or cytoplasmic conductivity in a high-throughput manner (Cheung et al. 2010; Gawad et al. 2001; Sun and Morgan 2010). Single cells are flowed through two microelectrodes in the microchannels, in which the impedance of cells at multiple frequencies is measured (Chen et al. 2015).

TEER values of a cellular monolayer are widely accepted parameters for quantifying the integrity of cellular barriers during growth and differentiation (Srinivasan et al. 2015a). *In vitro* models of cells cultured on semipermeable supports and TEER measurement are frequently used to evaluate the permeability and absorption of drugs/chemicals. Impedance spectroscopy is a more reliable

and accurate representation of TEER values than the Ohm's law method (Benson et al. 2013). However, these 2D models do not mimic the *in vivo* microenvironment.

Table1. Commercial conventional TEER measurement systems.

Product	**Physics principle**	**Electrodes**	**Company**
EVOM™®	Ohm's Law	STX2 and STX3	World Precision Instruments
EVOM2™	Ohm's Law	STX2 and STX3 STX100 family EndOhm chambers	World Precision Instruments
EVOM3™	Ohm's Law	STX2 and STX3 STX100 family EndOhm chambers	World Precision Instruments
REMS AutoSampler	Ohm's Law	REMS electrode on robotic arm	World Precision Instruments
Millicell® ERS Voltohmmeter	Ohm's Law	handheld electrodes	Electrical Resistance System
Ussing Chamber	Ohm's Law	chambers	Warner Instruments
Nevisense™ (EIS)	Impedance spectroscopy	Non-invasive electrodes micro invasive (spike) electrodes	SciBase AB
ECIS®	Impedance spectroscopy	ECIS®arrays	Applied BioPhysics

4 Advances in TEER measurement in organ-on-a-chip

Integrating TEER measurement systems into organ-on-chip models that can replicate native tissue *in vivo* microenvironments can assist us in obtaining more predictable and reliable results than routine 2D *in vitro* models. Recording TEER levels in such microfluidic devices that mimic the architecture, anatomy, and physiology of the biological barrier in the body is essential for increasing our knowledge about stem cell biology, regenerative medicine, and drug development (Shrestha et al. 2020). Modeling biological barriers inside organ-on-chips or here barrier-on-chip is a complex process involving different technologies, including micro/nanoscale manufacturing techniques, surface modification methods, biomaterials, and stem cell biology.

4.1 Organ-on-a-chip

Organ-on-a-chip systems can be used to simulate the physiology of native tissue or model the physiopathology of various disorders at the microscale level (Arık et al. 2018; Henry et al. 2017b;

Srinivasan et al. 2015b). These systems are mainly recruited to mimic *in vivo* conditions, which are more relevant than traditional *in vitro* models and provide a more coherent picture of organs (Elbrecht et al. 2016a; van der Helm et al. 2019b). They can also be easily integrated into automatic and robotic systems. Furthermore, a variety of sensors and (micro/nanoscale) multi-electrode arrays can be integrated into these systems to measure TEER of barrier models and increase our understanding of permeability and the integrity of barriers. Organ-on-chips can implement a variety of physical forces like shear stress, mechanical waves, and different pressures on cellular layers during TEER monitoring. In addition, they are transparent, and a variety of biological assays such as morphological studies can be simultaneously performed using time-lapse microscopy (Leung et al. 2022; Mencattini et al. 2019). These organs-on-chips can be equipped with the ability to be integrated with different types of TEER measurement equipment. The following section describes recent advances in TEER measurements inside organ-on-chips.

4.1.1 TEER measurement in kidney-on-a-chip

The kidney is one of the most vital organs for regulating body extracellular fluid volume, osmolarity, ion concentrations, and transferring waste and toxins to urine (Meijers et al. 2018). These procedures are not possible without precise coordination and proper function of the two cellular layers in the kidney, the renal tubular epithelial layers in nephron (including Bowman's capsule, proximal convoluted tube, the loop of Henle, and distal convoluted tubule), and the capillary endothelial layers (including glomerulus, and peritubular capillaries) that form active renal barriers for filtration (Lee et al. 2018). The transportation of different elements through the tubular epithelium directly depends on the modulation of tight junction permeability. The measurement of TEER in renal transporting epithelial cells can provide important information about the condition of tight junctions, monolayer formation, and permeability of cell layers in interaction with different drugs inside various kidney-on-a-chip models (Zanetti 2020).

Evaluating renal epithelial cells by measuring their TEER under physiologically relevant fluid flow conditions can reveal the impact of these drugs on cell barriers and renal microscale physiology. Ferrell *et al*. integrated TEER measurement electrodes in a bilayer microfluidic bioreactor to monitor proliferation, permeability, and the integrity of tight junctions between epitrichial cells under renal fluid flow and shear stress conditions. They were able to generate cell monolayers by culturing human renal epithelial cells (HREC) and Madin–Darby canine kidney (MDCK) epithelial cells inside this chip for TEER studies. They implemented Ag/AgCl (200 μm diameter) and Ag electrodes on each side of the membrane with an epoxy layer and connected them to an outside epithelial Volt/Ohm (EVOM2^{TM}) recorder. Their setup enabled them to measure changes in TEER during the formation of cellular barriers in response to shear stress and the disruption of tight junctions after the Ca^{2+} switch (Ferrell et al. 2010).

The proximal tubule is one of the most functional portions of the renal–blood barrier, which is highly vulnerable to drug toxicity (Maass et al. 2019). In a recent study, Yan's research team designed TEER electrodes for a proximal tubule-on-a-chip that simulated the topography and physiology of this part of renal tubules. This TEER system has potential to be integrated within channels mimicking the human proximal tubules in terms of geometries and fluid dynamics to monitor the integrity of the renal–blood barrier (Yan 2020). In another study, Asif *et al.* printed square-shaped indium tin oxide electrodes (4 mm × 500 nm) on glass chips using a screen-printing technique for measuring impedance changes in a proximal tubule-on-a-chip. They used this device for studying nephrotoxicity in proximal tubule models (Asif et al. 2020).

4.1.2 TEER measurement in eye-on-a-chip

The blood–retinal barrier is a restrictive biological barrier in the eye responsible for regulating the transportation of nutrients, waste, and other physiological metabolites between blood and the retina and plays a central role in maintaining homeostasis (Liu and Liu 2019). There are two types of

blood–retina barriers in the eyes: inner and outer. The inner blood–retinal barrier is made of non-fenestrated endothelium supported by astrocytes, Müller cells, and pericytes. The outer blood–retinal barrier is more permeable and made of pigment epithelium, located between the outer neural retina and fenestrated capillaries (Bird 2006).

TEER in retina-on-a-chip models provides a suitable approach to decipher retinal and neurovascular diseases for the identification of factors that affect this barrier's integrity, leading to blindness. In a recent study, Yeste *et al.* equipped a compartmentalized retina-on-a-chip with impedance analyzer TEER recording platinum electrodes (40 μm × 4 mm) for studying the cell barrier formations and interconnections of relevant cell layers in the outer blood–retinal barrier. Their platform was ideal for culture retinal endothelial cells, ganglion cells, and retinal pigment epithelium in parallel compartments to model the barrier. Furthermore, implementing a grid of microgrooves inside the chip improved paracrine signaling and heterotypic cell–cell interactions between different tissues constructing the barrier (Figure 3. a,b) (Yeste et al. 2018).

Bruch's membrane is a prominent part of the retina–blood barrier originally located between the retinal pigment epithelium and the fenestrated choroidal capillaries of the eye (Booij et al. 2010). Chen *et al.* integrated TEER electrodes into a two-layer multichannel microfluidic device that mimics the *in vivo* retinal pigment epithelial cells-Bruch's membrane-fenestrated choroids. The platinum electrode (diameter 300 μm) monitored the TEER of co-cultured human retinal pigmental epithelial cells and human umbilical vein endothelial cells with the assistance of an outside EVOM® instrument (Figure 3. c)(Chen et al. 2020).

The cornea is a transparent layer in the eye, which refracts the incoming light and acts as a chemical and mechanical barrier to prevent external substances from entering the eye. The cornea also has a significant absorption capacity for topical drugs as well as forming a tight barrier (Van Meenen et al. 2021). The cornea is a multilayered structure containing three main layers: epithelium,

stroma, and endothelium (Reichl 2008; Sarmento et al. 2012). Yu *et al.* developed a human cornea-on-a-chip by culturing immortalized human corneal epithelial and endothelial cells on the opposite sides of a collagen-coated porous membrane. The system was able to mimic the ocular surface of the eye by creating an air–liquid interface inside the chip. They adapted this device to host a TEER zone connected to a Millicell-ERS electrical resistance system to measure the integrity of cell layers (Yu et al. 2021).

4.1.3 TEER measurement in heart-on-a-chip

The response of electrically active tissue, for instance the myocardium to inflammation and different drugs, can be investigated inside perfusion endothelialized cardiac models, in which a thin membrane separates cardiomyocytes and endothelial cells (Zhao et al. 2020). The real-time monitoring of TEER in heart-on-a-chip models can predict the effect of various pharmacological-based treatments.

For studying electrical activity and the barrier function of the heart in pharmacodynamics studies, Maoz *et al.* implemented a multi-electrodes array and TEER electrodes to measure the field potential of cardiac cells and endothelial cell layers. They designed a complicated device to monitor the dynamic alterations of vascular permeability and cardiomyocyte function in interaction with some immunological factors such as an inflammatory stimulus mediated by tumor necrosis factor-alpha (TNF-α) and drugs (isoproterenol). Measurement of TEER after adding TNF-α showed a significant drop from 230 ± 45 Ω to 15 ± 13 Ω, which indicated severe damage to the tight junctions. They also observed that damaged F-actin polymerization by TNF-α causes endothelial dysfunction (Maoz et al. 2017).

4.1.4 TEER measurement in skin-on-a-chip

Skin is another biological barrier that plays an essential role in protecting internal organs against foreign pathogens, toxins, and other harmful agents. The integrity of this tissue is critical to

perform its functions (Cui et al. 2021). Various skin-on-a-chip models have been developed to simulate the epidermis microenvironment for *in vitro* skin toxicity, pharmacological, and cosmetic assays (Risueño et al. 2021). TEER measurement is considered a suitable option to investigate the effect of various factors on skin-on-a-chip models. Episkin and EpiDerm are two commercial *in vitro* skin-on-a-chip models that have been primarily used (Sutterby et al. 2020; Wufuer et al. 2016; Zhang et al. 2018). Alexander *et al*. developed a noninvasive automated sensor-based monitoring platform for measuring TEER in microfluidic-based reconstructed human epidermis. This commercial skin-on-a-chip, called an *in vitro* diagnostics system, can monitor microtissue properties such as the extracellular acidification rate and TEER of fibroblast cells cultured inside a chip based on an intelligent mobile laboratory for *in vitro* diagnosis (IMOLA-IVD, cellasys GmbH, Kronburg, Germany) (Figure 3.d). The IMOLA-IVD is a lab-on-a-chip assay system that consists of a power supply, analog and digital modules, and a BioChip with integrated sensors that passively monitor the microenvironment of the cellular model. The device with two automated fluidic systems also supported the culturing of more complicated biologic structures like EpiDerm™ human dermal tissue models (Alexander et al. 2018a).

4.1.5 TEER measurement in gut-on-a-chip

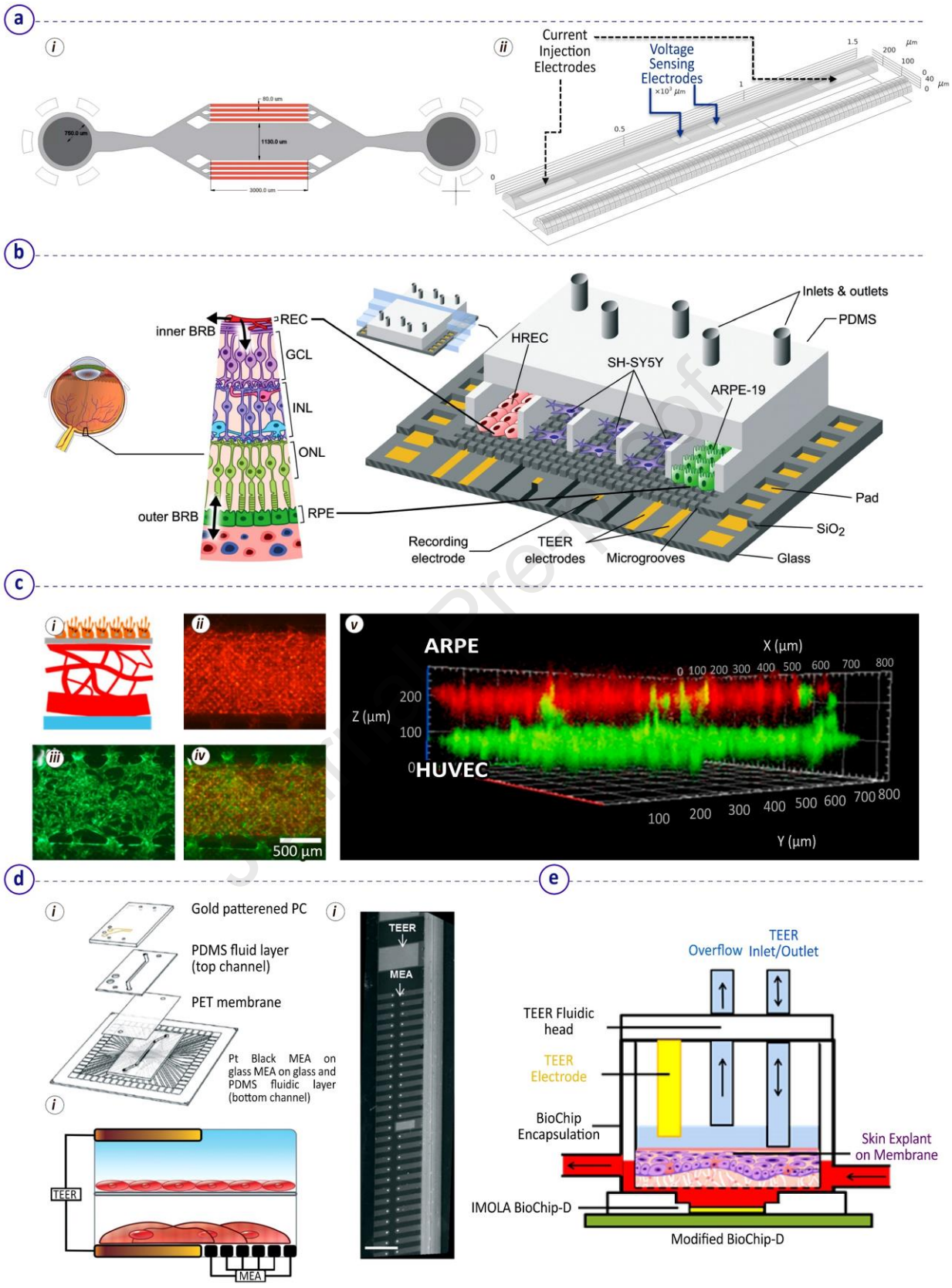
Another critical biological barrier in our body is the gastrointestinal system responsible for digesting foods and transporting nutrients to the vascular and lymphatic systems. Gut-on-a-chip models are miniaturized intestine models that are highly interested in drug discovery (Marrero et al. 2021).

Enhancing gut-on-a-chip models with TEER can advance our understanding of gut physiology and the development of new drugs. Van der Helm *et al*. investigated the transepithelial barrier function in human intestinal tract by integrating impedance spectroscopy and an electrical stimulating system inside a gut-on-a-chip. Their noninvasive sensing method allows researchers to monitor

the differentiation of human intestinal epithelium cultured towards villus microtissues; villus is a small vascular projection that increases the surface area of a membrane in the gastrointestinal system and is crucial for absorbing nutrients. For this aim, they embedded six electrodes inside the chip and connected to an Autolab PGStat (Metrohm Autlab B.V) to perform impedance spectroscopy measurements of the cell layer for 12 days (Figure 3. e-f) (van der Helm et al. 2019a). Studies showed a difference in the TEER measures of the same cell layers between microfluidic chips and trans-well systems caused by the geometry of the device. Odijk *et al*. invented a mathematical model to improve the fidelity of DC-based TEER measurements in a gut-on-a-chip device. Their model minimized the differences in measured TEER of the same cell layers between microfluidic chips and trans-well systems. They showed that the confined environment of microfluidic channels could cause a higher TEER than that in trans-well ones, and this difference needs to be considered for all biological studies. They found that the increase in TEER measured inside microfluidic chips is attributed to the natural 3D morphogenesis of the villus when the cells benefit from the persistent flow and simulated peristaltic motions on the chip (ávan der Meer et al. 2015). In another study, Bossink *et al*. integrated platinum electrodes into a microfluidic device through a cleanroom-free fabrication method to monitor the TEER in a gut-on-a-chip through impedance spectroscopy. They successfully observed and monitored this barrier's formation, disruption, and recovery, made of Caco-2 cell layers cultured on collagen-coated chips during three weeks (Bossink et al. 2021). Poenar and his team designed a sandwich ring device in which a specimen is clamped between two PDMS ring plates containing a central hole smaller than the sample. They indicated that the fresh specimen's TEER values in the incubator decreased by approximately 40% and reached approximately 190 $\Omega.cm^2$ after four days. Keeping the tissue at room temperature showed a more pronounced decrease; more than 75% reduction in the TEER

values was observed by the end of day four. Therefore, the physiological temperature is critical for preserving tissue viability for comprehensive assessments (Poenar et al. 2020).

With the largest surface within the body, the gastrointestinal tract's primary function is to control the absorption of nutrients and conduct them into the bloodstream (Elbrecht et al. 2016a; Sakolish et al. 2016b). Several studies have been carried out to mimic the gastrointestinal tract to analyze cell behavior and how the distribution of the normal flora can affect the viability and the function of the tissue (Lau et al. 2021). One of the *in vitro* gastrointestinal model limitations is the lack of living normal flora on the surface of cultured tissue. Bacterial flora controls the absorption and metabolism of the nutrients and, most importantly, barrier function. Disturbance in the flora can generate multiple diseases, particularly related to the control of immunity. Co-culturing *Lactobacillus rhamnosus GG* with the gut epithelium improves the barrier function, leading to 3–4-fold higher TEER values in microfluidic cultures compared with static cultures (Sakolish et al. 2016b).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ARPE-19
Inlets & outlets
PDMS
Pad
SiO2
Recording electrode
TEER electrodes
Microgrooves
Glass
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ARPE
HUVEC
X (µm)
Y (µm)
Z (µm)
500 µm
d
e
Gold patterened PC
PDMS fluid layer (top channel)
PET membrane
Pt Black MEA on glass MEA on glass and PDMS fluidic layer (bottom channel)
TEER
MEA
TEER Overflow Inlet/Outlet
TEER Fluidic head
TEER Electrode
BioChip Encapsulation
Skin Explant on Membrane
IMOLA BioChip-D
Modified BioChip-D

Figure 3. a) Microfluidic circuit geometries designed for a kidney-on-a-chip device containing 4-electrode TEER. Reproduced with permission from (Yan 2020). b) Cell layers of the retina, from the anterior to the posterior part of the retina: retinal endothelial cells (REC), ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL), and retinal pigment epithelium (RPE). Double arrows indicate the transport crossing the inner and outer blood-retinal barrier (oBRB) Reproduced with permission from (Yeste et al. 2018). c) Schematic representation of the presented microfluidic cell culture device, including cell arrangement used in the experimental setup. Endothelial (Human Research Ethics Committees, HRECs) and epithelial (a spontaneously arising retinal pigment epithelia (ARPE)-19) cells are located at the end compartments where TEER measurement electrodes and SH-SY5Y cells are located in the middle compartments potential extracellular recording electrodes. Reproduced with permission from (Chen et al. 2020). d) The oBRB model prototype was built using a microfluidic device. i) Schematic showing the anatomical structure of oBRB (retinal pigment epithelial cells–Bruch membrane-choroid). ii-iv) tri-culture of ARPE–human umbilical vein endothelial cells (HUVEC)-human lung fibroblasts (NHLF). ii) ARPE cells stained in red were cultured in the upper channel. iii) GFP-HUVEC microvessels were cultured in the lower channel. iv) Overlay of ii) and iii). v) Confocal microscopic view of the cross-section of the co-culture model. Reproduced with permission from (Maoz et al. 2017). e) Schematic diagrams of modified BioChip with TEER fluidic head. The fluidic system with the medium is highlighted in red. IMOLA: Intelligent mobile lab. Reproduced with permission from (Alexander et al. 2018b).

4.1.6 TEER measurement in blood–brain barrier-on-a-chip

The blood–brain barrier controls brain homeostasis and keeps the brain isolated from biological agents, drugs, and toxic materials present in capillary vessels (Lau et al. 2021). The behavior of blood–brain barrier models inside microfluidic devices under different conditions using TEER electrodes for studying neurodegenerative diseases has been investigated in a limited number of studies. For instance, Griep *et al*. inserted platinum electrodes inside a microfluidic chip to monitor the TEER of endothelial monolayers inside the blood–brain barrier-on-a-chip model using the impedance spectroscopy method. The human brain endothelial cells were cultured inside this microscale bioreactor for seven days. Their TEER was measured using impedance spectroscopy while cells were subjected to shear stress and the inflammatory cytokine TNF-α. Their study showed that treating barriers with TNF-α resulted in a 10-fold reduction in TEER (Griep et al. 2013a).

Van der Helm *et al*. believed that integrating electrodes inside microfluidic chips near cellular barriers could significantly reduce the visualization of cells. So they designed a TEER measuring system inside a blood–brain barrier-on-a-chip, made of four Platinum wire electrodes inserted far from the cellular barrier (van der Helm et al. 2016). Douville *et al.* incorporated TEER electrodes for *in vitro* measurement of the blood–brain barrier permeability in a real-time manner. They cultured mouse brain-derived endothelial cells on the membrane of a microfluidic device and observed an increase in TEER values, validating the establishment of tight junctions. Their results showed that the introduction of TritonX-100 led to a decrease in TEER levels (Douville et al. 2010). Brown *et al*. studied the permeability under stress inside a blood–brain barrier-on-a-chip made of primary human brain-derived microvascular endothelial cells, primary pericytes, astrocytes, and human iPSC-derived neurons. They found that cold shock, decreased nutritional perfusion, and neuro-inflammation all resulted in a decline in TEER values, showing the vulnerability of the blood–brain barrier under these experimental conditions (Brown et al. 2016). Booth *et al.* developed a four-layered microfluidic system for culturing endothelial cells astrocytes and modeling a blood–brain barrier-on-a-chip facilitated with a thin polycarbonate culture membrane and AgCl thin-film TEER electrodes. Their study demonstrated that exposure of the blood–brain barrier to histamine temporarily decreases the TEER following the concise formation of trans-endothelial gaps and raised trans-cytosis (Booth and Kim 2012). In another study, Badiola-Mateos *et al.* implemented a multifrequency TEER sensor array in a blood–brain barrier-on-a-chip to monitor the integrity of endothelial and pericytes cells layers through machine learning algorithms (Badiola-Mateos et al. 2021).

4.1.7 TEER measurement in capillary-on-a-chip

The capillary endothelial layer permanently interacts with a stream of different blood cells such as red blood cells, neutrophils, lymphocytes, and platelets. These barriers need to have high electrical

resistance to keep their integrity and functionality and prevent stroke in different organs, including the heart and the brain (Mcelroy et al. 2021). Therefore, integrating TEER measuring systems inside microfluidic devices simulating both *in vivo* capillary environments and circulation of blood cells can be helpful for a more detailed study of drug permeability through different organs. In this instance, Vogel *et al*. performed TEER-based measurements using square wave pulses in a microfluidic device in which an endothelial layer was exposed to a stream of red blood cells (Vogel et al. 2011).

4.1.8 TEER measurement in lung-on-a-chip

The main task of the lung blood–air barrier is to prevent air bubbles from forming in the blood capillary and, conversely, blood/plasma from accessing pulmonary alveoli (Awad and Aljebali 2021). This barrier also regulates tissue homeostasis and airway stability. Furthermore, this barrier secretes surfactants to keep airways wet and prevents harmful particulates from damaging the pneumocytes (Shrestha et al. 2020). Traditional *in vitro* lung models have been based on trans-well cultures to simulate lung barriers. Microfluidics devices currently include an air-filled upper layer with gas exchange capability and a vascular chamber to mimic the capillary network. Henry *et al*. developed a TEER measuring system by implanting electrodes made of gold-patterned polycarbonate and a polyester membrane in human lung-on-a-chip. They investigated the capacitance of the human pulmonary epithelial cell layer using 4-point impedance measurements at varying frequencies. A comparison of the TEER values measured from cell layers in culture medium or at the air–liquid interface showed that the average increase in TEER values was higher at the air–liquid interface (Henry et al. 2017b).

4.1.9 TEER measurement in reproductive tract-on-a-chip

The oviduct and its covering epithelium provide an environment for the transportation, maturation, and fertilization of gametes (Almiñana and Bauersachs 2020). Developing microfluidic-based *in*

vitro methods for studying oviducts can enhance the differentiation of stem cells or extended culture periods. The TEER value of oviduct epithelial cells is an essential factor that provides important information about the culture quality, permeability, and tightness of junctions. Ferraz *et al*. developed an oviduct-on-a-chip system to investigate the *in vitro* genome reprogramming of zygotes. They cultured bovine oviduct epithelial cells on a porous membrane embedded inside a microfluidic device. Their system contained two independent and perfusable compartments, mimicking the circulating hormone changes during the peri-ovulation period. The cell layers on the chip model exhibited high potency in the growth and development of embryos comparable with *in vivo* models. They simulated the luteal-phase simulation and pre-ovulatory phase hormone conditions and studied the TEER of cell layers in response to these hormonal changes. Furthermore, the amount of DNA methylation in zygotes, which is a challenge in reproductive biology, on this chip is lower than conventional *in vitro* models (Ferraz et al. 2018).

4.1.10 TEER measurement in liver-on-a-chip

The liver is one of the vital organs in the human body, playing different roles in human body such as metabolism, the immune system, hemostasis, digestion, and detoxification. Hepatocytes, hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells are major cells in the liver. Tight junctions between hepatocytes and a type of hepatic epithelial cells called cholangiocytes in canaliculus and act as a barrier to blood and bile diffusion (blood bile barrier) by sealing the lumen of bile canaliculi between adjacent hepatocytes. The integrity and functionality of these structures is are essential for preserving the polarity and overall functionality of the liver (Pradhan-Sundd and Monga 2019).

In hepatic fibrosis, the hyper activation of hepatic stellate cell results in excessive ECM deposition in the liver. The progression of hepatic fibrosis can be performed in real-time manner to the in vitro models. Farooqi *et al.* integrated electrical and photoelectric sensors for real-time monitoring

of physiological symptoms in 2D HepG2 monolayer cell sheets produced by doxorubicin, epirubicin, and lapatinib in a liver-on-a-chip device. They utilised indium tin oxide electrodes (located at the bottom and top of microchannels) to assess TEER and evaluate drug-induced disruption in cell-to-cell tight junctions. In addition, their system included a photoelectric sensor for monitoring the pH and acidity of the cell culture fluid. In addition, they confirmed impedance-based relative cytotoxicity results by evaluating albumin and lactate levels and cell-cell tight junction formation (E-cadherin imaging in confocal microscopy) (Farooqi *et al.* 2020).

Chethikkattuveli Salih *et al.* investigated the influence of ECM types on liver tissue development utilising an intensity-based image processing technique, TEER sensors, and hepatic metabolic quantification. The TEER and image processing data were utilised to evaluate the development of a microfluidic liver for modelling the physiology of the human liver. According to their findings, Matrigel and fibronectin were the most relevant ingredients for developing liver-on-a-chip systems (Chethikkattuveli Salih *et al.* 2021). In another study, the effect of different concentrations of foetal bovine serum (FBS) on tight junction formation in gut, liver, and kidney-on-a-chip devices was monitored by measuring the transendothelial electrical resistance (TEER) and it was determined that 5% is the optimal concentration (Salih et al. 2020).

Farooqi *et al.* created a liver fibrosis-on-chip model for measuring TEER in real-time during fibrosis progression. Regarding this, HepG2 cells and fibroblasts were co-cultured and treated with transforming growth factor 1 (TGF-1) to induce fibrosis. They embedded TEER electrodes (created using chemical vapour deposition) and reactive oxygen species (ROS) sensors (created using solution-based inkjet printing) on the surface of glass and monitored the effects of TGF-1 on fibrosis (Farooqi et al. 2021). TEER systems also have been implemented in hepatic tumor-on-a-chip devices for studying the effect of anticancer agents such as *Acer cappadocicum Gled* (Farooqi et al. 2022).

Table 2. TEER measurement systems of biological barriers inside organ-on-chip devices.

Organ	Mimicry conditions	Cells	TEER measurement system	Ref
Kidney	- Bilayer device - Renal fluid flow and shear stress	HREC MDCK	- Ag electrodes - Ohm-based, EVOM2™	(Ferrell et al. 2010)
	- *In silico* modelling of geometries and fluid dynamics of human proximal tubules	HK-2 cells	Impedance-based	(Yan 2020)
	- Proximal tubule-on-a-chip, proximal shear stress, - TEER and pH measurement, glass device	HK-2 Fibroblasts	- Indium tin oxide electrodes - Impedance measurements	(Asif et al. 2020)
Eye	- Blood–retinal barrier on a chip Parallel compartments for co-culture system	HREC SH-SY5Y ARPE-19	- Grid of microgrooves and metal electrodes - Impedance-based	(Yeste et al. 2018)
	- Retinal pigment epithelial cells-Bruch's membrane-fenestrated choroids	HUVECs ARPE-19	- Platinum electrode - Ohm-based, EVOM®	(Chen et al. 2020)
	- A human cornea-on-a-chip - Air-liquid interface for mimicking ocular surface	hCEpi hCEnd	- Holes inside device for Millicell-ERS	(Yu et al. 2021)
Heart	- Mimicking the vascular administration of isoproterenol and TNF-α - Applying shear stress on cardiomyocytes	HUVECs Cardiomyocytes	- Multi-electrode arrays and electrodes - Impedance-based	(Maoz et al. 2017)
Skin	- Human epidermis models - Air-liquid interface - Automated fluidic systems	L929	- Wire and planar electrodes - Impedance-based - IMOLA-IVD interdigitated electrode sensors	(Alexander et al. 2018a)
Gut	- Gut-on-a-chip - Applying shear stress	Caco-2	- Mathematical model for differences measured in TEER between chips and Transwell systems - Ag/AgCl electrodes, Ohm-based, EVOM2™	(ávan der Meer et al. 2015)
	- Human intestinal epithelium in a gut-on-a-chip	Caco-2	- Semi-transparent gold electrodes - Impedance-based, Autolab PGStat	(van der Helm et al. 2019a)
	- Human intestinal epithelium in a gut-on-a-chip	Caco-2	- Platinum wire electrodes - Impedance-based - Zurich instruments HF2IS impedance spectroscope	(Bossink et al. 2021)
	- Esophageal epithelium sandwiched by the two half-chambers through a chip in physiological temperature	Mucosal/Epitheliu m Layer from the Porcine Esophagus	- Chopstick electrodes - Ohm-based, EVOM2™	(Poenar et al. 2020)

Blood–brain barrier	- Blood-brain-barrier-on-a-chip - Two-layer channel	MDCK-2, C2C12, b.End.3	- Ag/AgCl electrodes - Impedance-based, Autolab Potentiostat/Galvanostat	(Douville et al. 2010)
	- Four-layered chip - Applying shear stress effects on endothelial cells	b.End.3 C8D1A	- AgCl thin-film TEER electrodes - Ohm-based, EVOM2^{TM}	(Booth and Kim 2012)
	- Blood-brain-barrier-on-a-chip - Applying shear stress on cell layer	hCMEC/D3	- Platinum electrodes, Impedance-based - Hp4194a impedance/gain phase analyser	(Griep et al. 2013a)
	- Blood-brain-barrier-on-a-chip	CMEC/D3	- Four platinum wire electrodes - HP4194A impedance/gain phase analyser	(van der Helm et al. 2016)
	- Mimicking responses to inflammatory stimulation on a chip - Applying shear stress on neurons	hBMVECs hiPSC-derived Cortical Neurons Astrocytes	- Impedance-based - Custom-built multi-frequency impedance analyser	(Brown et al. 2016)
	- Multi-layer microfluidic platform Co-culture system	hCMEC/D3 Pericytes	- Array of concentric interdigitated gold electrodes - Multi-frequency TEER - Electric impedance spectroscopy (EIS) - Machine learning algorithms for analysing	(Badiola-Mateos et al. 2021)
Capillaries	- Flowing stream of red blood cells - Applying shear stress on endothelial cells	bPAEC RBC	- Ohm-based TEER	(Vogel et al. 2011)
Lung	- Air–liquid interface - Lung-on-a-chip	hAECs	- Gold-patterned electrodes - 4-point impedance measurements, PGSTAT128N (Metrohm Autolab BV)	(Henry et al. 2017b)
Reproductive system	- Oviduct-on-a-chip - Applying shear stress on epithelial layer	Epithelial cells Gametes, Embryos	Ag/AgCl wire electrodes, Millicell, USA	(Ferraz et al. 2018)
Liver	- Liver-on-a-chip - Applying shear stress on cells	HepG2	- Indium tin oxide electrodes, impedance-based. - Photoelectric pH sensors	(Farooqi et al. 2020)
	- Liver, gut, kidney on a chip - Applying shear stress on cells	Caco-2 HepG2 HK2	- Indium tin oxide electrodes, impedance-based	(Salih et al. 2020)
	- Liver-on-a-chip - Implementing different ECMs: Matrigel, collagen, fibronectin, and poly-L-lysine	HepG2	- Indium tin oxide electrodes, impedance-based	(Chethikkattuveli

				Salih et al. 2021)
	- Liver fibrosis-on-chip - TGF-β effect on liver fibrosis - Co-culture system, - Applying shear stress on cells	HepG2 Hs68	- Indium tin oxide electrodes, impedance-based	(Farooqi et al. 2021)
	- Hepatic tumor-on-a-chip - Therapeutic effect of methanolic extracts of *acer cappadocicum gled*	HepG2	- TEER sensor - Impedance-based	(Farooqi et al. 2022)

Abbreviations: HREC: Human Retinal Endothelial Cells, MDCK: Madin-Darby canine kidney, HK2: Human Kidney 2, HUVECs: Human Umbilical Vein Endothelial Cells, HCEpiC: Human Corneal Epithelial Cells, HCEnd: Human Corneal Endothelial Cells, Caco-2: human colorectal adenocarcinoma, b.End.3: mouse brain endothelial cell line, C8D1A: mouse neuronal astrocyte cell line. C8D1A: brain microvascular endothelial cell, hCMEC/D3: Human Cerebral Microvascular Endothelial Cell Line, hBMVECs: human Brain Microvascular Endothelial Cells, hiPSC: human induced Pluripotent Stem Cell, bPAEC: bovine Pulmonary Artery Endothelial Cells, RBC: Red Blood Cell.

5 Challenges and future perspectives

TEER measurement is a potential approach for studying the behavior of biological membranes in different diseases. This parameter can be assessed *in vivo* for diagnostic purposes or in vitro to examine the effect of various medications on (healthy or pathologic) barrier models. TEER can provide precise information on the ability of biological barriers to cope with immune cells, drugs, nanoparticles, and biomolecules. Organ-on-a-chip is a promising technology that enables the miniaturization of different biological barriers and can be integrated with TEER measuring systems. This review evaluates the state-of-the-art advances in the measurement of the TEER of various biological barriers modeled inside organ-on-chips.

Four generations of TEER measurement systems have been developed during the past decade (Figure 4). The first-generation instruments operated based on the Ohm law and utilized manual or fixed electrodes implemented inside Endohm and using chambers. The significant advantages of impedance-based techniques for TEER measurements led to the flourishing of the second-generation instruments for the measurement of TEER Applied BioPhysics (ECIS®) Cultureware. These devices were equipped with user-friendly disposable electrodes for measuring TEER with high precision. However, the culture was in a static condition, which was undesirable for biologic applications. Therefore, Applied BioPhysics recently introduced a third generation of TEER measurement systems, ECIS® Flow Array µ-slides, to the market that can produce dynamic flows inside simple channels of approximately 700 µm in height. This generation is assisted with disposable electrode arrays with different patterns but cannot mimic the biological barriers structure. The last generation of TEER measurement systems, which are not still on the market, integrate TEER measurements systems with organ-on-chips. These systems can be integrated with AC- and DC-based systems and produce better dynamic flow conditions than previous chambers,

culture wares, and channel µ-slides. Furthermore, these systems can be enriched with different cell layers, mimicking barriers' complexity and providing cells with different culture mediums, drugs, conditions, and even air flow. The cells inside these systems interact with lower amounts of fluids, tensions and micro/nanopatterned electrodes can be integrated into the systems during the manufacturing processes (Figure 4).

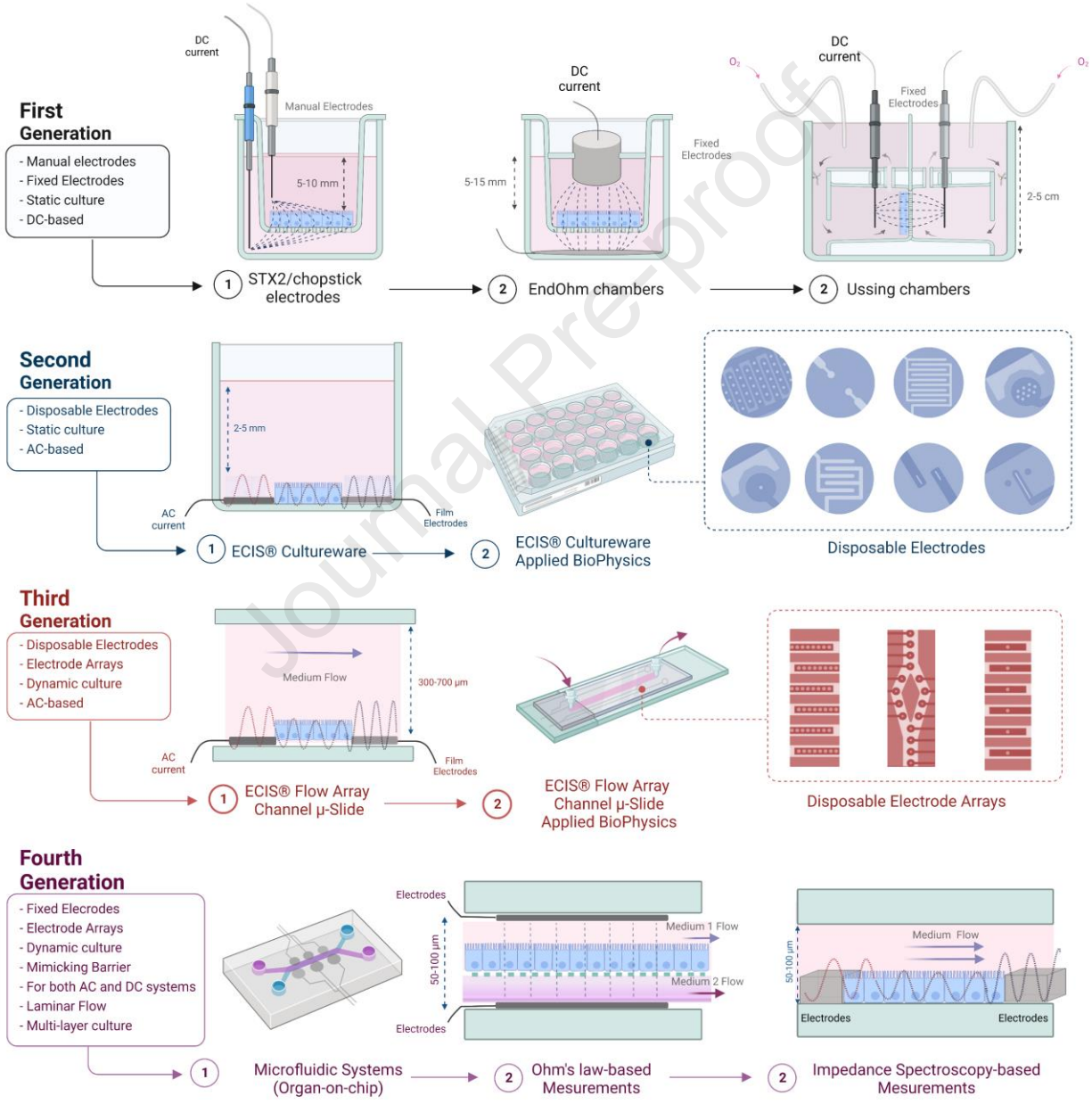

Figure 4. The schematic shows the development of TEER measurement systems. The STX2 Chopstick Electrodes, EndOhm, Ussing chambers, ECIS® Culturewares, and ECIS® Flow Array µ-slides were commercially developed sequentially. The new TEER measurement systems are based on microfluidic technology and organ-on-chips.

The development of organ-on-a-chip technology for TEER measurement purposes is highly dependent on advanced technologies such as microfabrication, nanotechnologies, and surface modification (Brown et al. 2016; Cochrane et al. 2019; Kennedy et al. 2021; Santa-Maria et al. 2021; Soucy et al. 2019). They can assist biomedical researchers in implementing TEER measurement by incorporating synthesized circuits, connectors, sensors, electrodes, and other electronic micro/nanostructures within the organ-on-chips (Asif et al. 2020; Egunov et al. 2021; Gopinath et al. 2019; Maoz et al. 2017; Wu et al. 2020b). In this regards, one of the most important factors in increasing the accuracy of TEER measurement is the fabrication and proper integration of different electrodes (excitation, recording, etc.) inside organ-on-a-chip devices (Srinivasan et al. 2015a). These electrodes can be fabricated using metallic elements (gold, platinum, copper, silver) (Ferrell et al. 2010; Yeste et al. 2018) in different shapes (cylindrical (Ferrell et al. 2010), rectangular (Yeste et al. 2018), nanofilms (Asif et al. 2020), wires (van der Helm et al. 2016), micro/nanoarrays (Maoz et al. 2017)) and sizes through various manufacturing methods and embedded inside chips (Pallarola et al. 2017). The TEER electrodes in the first studies were simple in the form of thin wires or plates that were directly inserted into a microfluidic chip. In the past few years, complex electrodes in the shape of multi-electrode arrays have been printed on glass surfaces using different methods such as physical vapor deposition and e-beam evaporation and integrated into organ-on-a-chip systems (Bourg et al. 2019; Soum et al. 2019; Yeste et al. 2018) This generation of electrodes significantly decreased the movement of electrodes inside the device, increased accuracy and repeatability, and decreased the undesired noise in TEER studies. The undesired mobility of electrodes within cell culture channels/chambers is a big challenge that can

result in non-reproducibility of TEER measurement values. These electrode arrays can be easily integrated with electric circuits and other sensing electrodes (Ferrari et al. 2020). The advances in electrically conductive polymers and nanoparticles in the last few years have introduced transparent electrodes that significantly improve the imaging quality, which is highly valuable for cellular studies. The flexible electrodes can assist in mimicking the movement ability of devices, which are vital in some organs such as the lungs and heart (McCullough 2020; Wang et al. 2020a). These electrodes create a uniform current density while reducing noise. Also, they can be applied to organ-on-a-chip from a simple single-channel model to complex models toward those trying to mimic multi-organ and human-on-a-chip.

Advances in microfabrication technologies have had a significant affect in the field of TEER. Conventional fabrication methods include photolithography for the design of planar microchannels. These channels are mainly produced by molding a soft polymer on a silicon wafer, resulting in planar microchannels. However, design complexity leads to the generation of complex microchannels fabricated through multi-layer lithography. This method results in the generation of nonplanar microchannels at the cost of increased fabrication time. More importantly, the mold fabrication process requires the use of skillful users and advanced facilities. These challenges have force investigators to try other possibilities. 3D printing or additive manufacturing are ideal candidates for the development of 3D microchannels, which can closely replicate an organ's complexity. This technique can create 3D structures by layer-by-layer formation of different materials. During the past decade, a variety of 3D printing techniques have been developed, including extrusion, droplet, selective laser sintering, stereolithography, inkjet 3D printing, laser-assisted printing, and selective laser melting (Pranzo et al. 2018). Integration of MEMS with additive manufacturing further advances the field toward the development of a new generation of

TEER platforms. Lately, many research groups have made increasing use of bioprinting or 3D bioprinting. Indeed, bioprinting is an extended use of additive manufacturing involving the generation of a tissue or organ through a bottom-up approach. The main aim is to closely mimic the cellular architecture. It has been envisaged that the recent advancement of microfabrication techniques will significantly revolutionize the measurement of TEER (Figure 5).

In addition to the presented milestone in electrode design, the fabrication and embedding of such micro/nanoscale structures is costly and requires cleanroom facilities, urging research teams to develop more cost-effective methods (Fan 2018; Karanassios 2018) (Bossink et al. 2021). Many opportunities in nanotechnology in developing electrodes are yet to be explored for their improvement. For instance, incorporating the diblock co-polymer micelle nanolithography technique and photolithography allows the fabrication of nanostructured nanoparticle-based patterned sensors (Meghani et al. 2020; Pallarola et al. 2017). Surface nanopatterning using photolithography and metal sputtering techniques can be utilized for surface functionalization of a substrate using metallic nanoparticles to study cell–substrate adhesion.

Different properties of membranes such as the selection of material, pore size, hydrophilicity, surface modification, and nanotopography play essential roles in the simulation of the basement membrane of biological barriers, which can be achieved by nanofabrication techniques (He et al. 2020; Nthunya et al. 2019). The origin of materials (natural or synthetic) implemented in a membrane can directly affect the formation and electrical behavior of cell layers (Pasman et al. 2020; Quirós-Solano et al. 2018; Schneider et al. 2021). In addition, the surface of the membrane can be modified using a variety of proteins such as albumin or ECM proteins such as fibronectin, laminin, and collagen (Peng et al. 2020; van der Helm et al. 2019a). Despite significant advances in TEER measuring technology, incorporating TEER measurement systems into organ-on-chip

technologies still presents certain obstacles. For instance, the design and integration of TEER measurement systems in multi-layer organ-on-a-chip devices for simultaneous measurement of TEER on the apical and basal sides of the membranes need to be optimized and standardized.

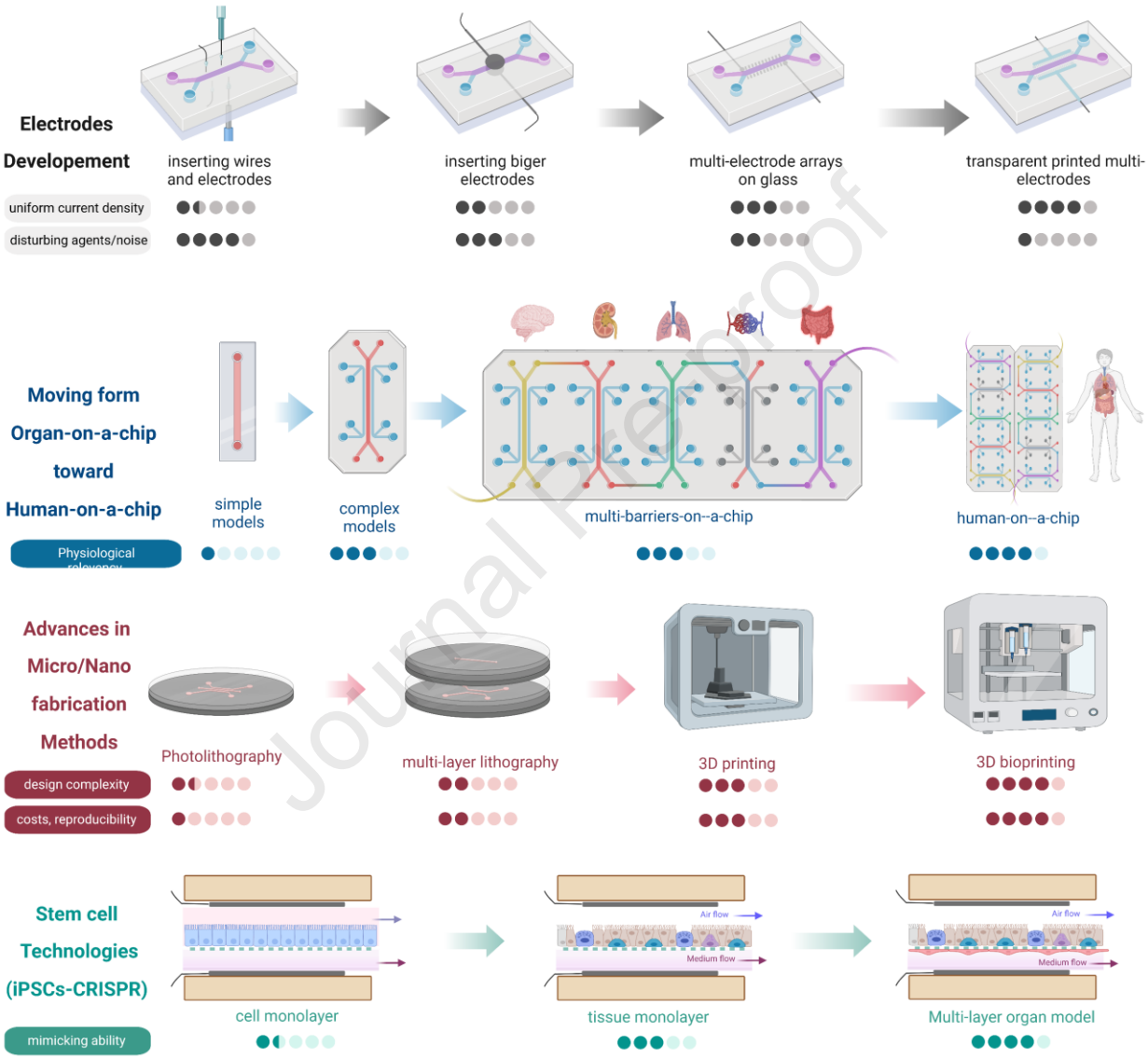

Figure 5. Electrode development, organ-on-a-chip platform complexity, microfabrication techniques, and stem cell technologies directly impact TEER's advancement. The research trend is the use of transparent printed multi-electrodes on complex multi-barriers-on-a-chip for multi-organ-on-a-chip in a multi-layer organ model engineered through advanced microfabrication technologies.

6 Conclusion

Microfluidics, membrane technology, and stem cell biology play a prominent role in designing an acceptable organ-on-a-chip device that can mimic specific barrier properties for TEER measurement purposes . The development of advanced fabrication methods such as 3D printing has changed the appearance of microfluidic organ-on-a-chip devices during the last decade. The advent of multilayered microfluidic systems integrated with electrodes significantly decreases the impact of disturbing agents in TEER measuring systems. Furthermore, more complexity and intricacy levels can be implemented in chips to mimic the native barrier. The integration of organ-on-a-chip with other sensors (such as oxygen, nitrogen), biosensors (such as glucose, hormones), and nanobiosensors can provide other valuable information about tissue barriers. In addition, the integration of microfluidic-based high throughput devices can assist the study of relationships between TEER changes with cellular, molecular, genomic, and next-generation sequencing studies. This advantage can expand our knowledge about complex signaling pathways related to TEER changes and cellular integrity.

7 Declaration of competing interest

The authors declare no conflict of interests in this research.

8 References

Aberg, P., Nicander, I., Hansson, J., Geladi, P., Holmgren, U., Ollmar, S., 2004. IEEE Trans. Biomed. Eng. 51(12), 2097-2102.
Alexander, F.A., Eggert, S., Wiest, J., 2018a. Genes 9(2), 114.
Alexander, F.A., Eggert, S., Wiest, J., 2018b. Genes (Basel) 9(2).
Almiñana, C., Bauersachs, S., 2020. Theriogenology 150, 59-69.
Amini, M., Hisdal, J., Kalvøy, H., 2018. Journal of Electrical Bioimpedance 9(1), 142.
Antimisiaris, S., Marazioti, A., Kannavou, M., Natsaridis, E., Gkartziou, F., Kogkos, G., Mourtas, S., 2021. Adv. Drug Del. Rev.
Arık, Y.B., van der Helm, M.W., Odijk, M., Segerink, L.I., Passier, R., van den Berg, A., van der Meer, A.D., 2018. Biomicrofluidics 12(4), 042218.
Asif, A., Kim, K.H., Jabbar, F., Kim, S., Choi, K.H., 2020. Microfluid. Nanofluid. 24, 1-10.

Augustine, R., Aqel, A.H., Kalva, S.N., Joshy, K., Nayeem, A., Hasan, A., 2021. Transl. Oncol. 14(7), 101087.
ávan der Meer, A.D., JungáKim, H., ávan der Helm, M.W., den Berg, A., 2015. Lab on a Chip 15(3), 745-752.
Awad, K., Aljebali, N., 2021.
Badiola-Mateos, M., Di Giuseppe, D., Paoli, R., Lopez-Martinez, M.J., Mencattini, A., Samitier, J., Martinelli, E., 2021. Sensors Actuators B: Chem. 334, 129599.
Bagchi, S., Chhibber, T., Lahooti, B., Verma, A., Borse, V., Jayant, R.D., 2019. Drug Des. Devel. Ther. 13, 3591.
Benson, K., Cramer, S., Galla, H.-J., 2013. Fluids and Barriers of the CNS 10(1), 1-11.
Bird, A.C., 2006. Retina, 971-977.
Booij, J.C., Baas, D.C., Beisekeeva, J., Gorgels, T.G., Bergen, A.A., 2010. Prog. Retin. Eye Res. 29(1), 1-18.
Booth, R., Kim, H., 2012. Lab Chip 12(10), 1784-1792.
Bossink, E.G., Zakharova, M., De Bruijn, D.S., Odijk, M., Segerink, L.I., 2021. Lab on a Chip 21(10), 2040-2049.
Bourg, S., Griveau, S., d'Orlyé, F., Tatoulian, M., Bedioui, F., Guyon, C., Varenne, A., 2019. Plasma Processes and Polymers 16(6), 1800195.
Brown, B.H., Milnes, P., Abdul, S., Tidy, J.A., 2005. BJOG 112(6), 802-806.
Brown, J.A., Codreanu, S.G., Shi, M., Sherrod, S.D., Markov, D.A., Neely, M.D., Britt, C.M., Hoilett, O.S., Reiserer, R.S., Samson, P.C., 2016. J. Neuroinflammation 13(1), 1-17.
Buchroithner, B., Mayr, S., Hauser, F., Priglinger, E., Stangl, H., Santa-Maria, A.R., Deli, M.A., Der, A., Klar, T.A., Axmann, M., 2021. ACS nano 15(2), 2984-2993.
Chaing, Y.-Y., Tu, K.-H., 2019. 2019 IEEE 14th International Conference on Nano/Micro Engineered and Molecular Systems (NEMS), pp. 351-354. IEEE.
Chatterjee, B., Gorain, B., Mohananaidu, K., Sengupta, P., Mandal, U.K., Choudhury, H., 2019. Int. J. Pharm. 565, 258-268.
Chen, J., Xue, C., Zhao, Y., Chen, D., Wu, M.-H., Wang, J., 2015. Int. J. Mol. Sci. 16(5), 9804-9830.
Chen, L.-J., Raut, B., Nagai, N., Abe, T., Kaji, H., 2020. Micromachines 11(1), 79.
Chethikkattuveli Salih, A.R., Hyun, K., Asif, A., Soomro, A.M., Farooqi, H.M.U., Kim, Y.S., Kim, K.H., Lee, J.W., Huh, D., Choi, K.H., 2021. Polymers 13(17), 3016.
Cheung, K.C., Di Berardino, M., Schade-Kampmann, G., Hebeisen, M., Pierzchalski, A., Bocsi, J., Mittag, A., Tárnok, A., 2010. Cytometry Part A 77(7), 648-666.
Cochrane, A., Albers, H.J., Passier, R., Mummery, C.L., Van Den Berg, A., Orlova, V.V., van der Meer, A.D., 2019. Adv. Drug Del. Rev. 140, 68-77.
Cojocaru, F.-D., Botezat, D., Gardikiotis, I., Uritu, C.-M., Dodi, G., Trandafir, L., Rezus, C., Rezus, E., Tamba, B.-I., Mihai, C.-T., 2020. Pharmaceutics 12(2), 171.
Cong, Y., Han, X., Wang, Y., Chen, Z., Lu, Y., Liu, T., Wu, Z., Jin, Y., Luo, Y., Zhang, X., 2020. Micromachines 11(4), 381.
Cui, M., Wiraja, C., Zheng, M., Singh, G., Yong, K.T., Xu, C., 2021. Advanced Therapeutics, 2100138.
Daza, P., Olmo, A., Canete, D., Yufera, A., 2013. Sensors Actuators B: Chem. 176, 605-610.
DePaola, N., Phelps, J.E., Florez, L., Keese, C.R., Minnear, F.L., Giaever, I., Vincent, P., 2001. Ann. Biomed. Eng. 29(8), 648-656.
Douville, N.J., Tung, Y.-C., Li, R., Wang, J.D., El-Sayed, M.E., Takayama, S., 2010. Anal. Chem. 82(6), 2505-2511.
Ebrahimi, Z., Talaei, S., Aghamiri, S., Goradel, N.H., Jafarpour, A., Negahdari, B., 2020. IET nanobiotechnology 14(6), 441-448.
Egunov, A.I., Dou, Z., Karnaushenko, D.D., Hebenstreit, F., Kretschmann, N., Akgün, K., Ziemssen, T., Karnaushenko, D., Medina-Sánchez, M., Schmidt, O.G., 2021. Small 17(5), 2002549.
Elbakary, B., Badhan, R.K.S., 2020. Sci. Rep. 10(1), 3788.

Elbrecht, D.H., Long, C.J., Hickman, J.J., 2016a. Journal of Rare Diseases Research & Treatment 1(1), 1.
Elbrecht, D.H., Long, C.J., Hickman, J.J., 2016b. Journal of Rare Diseases Research & Treatment 1(3).
Elliott, R., He, M., 2021a.
Elliott, R.O., He, M., 2021b. Pharmaceutics 13(1), 122.
Fan, Y., 2018. Micro & Nano Letters 13(10), 1367-1372.
Farooqi, H.M.U., Kang, B., Khalid, M.A.U., Salih, A.R.C., Hyun, K., Park, S.H., Huh, D., Choi, K.H., 2021. Nano Convergence 8(1), 1-12.
Farooqi, H.M.U., Khalid, M.A.U., Kim, K.H., Lee, S.R., Choi, K.H., 2020. Journal of Micromechanics and Microengineering 30(11), 115013.
Farooqi, H.M.U., Sammantasinghar, A., Kausar, F., Farooqi, M.A., Chethikkattuveli Salih, A.R., Hyun, K., Lim, J.-H., Khalil, A.A.K., Mumtaz, A.S., Choi, K.H., 2022. Life 12(2), 135.
Ferrari, E., Palma, C., Vesentini, S., Occhetta, P., Rasponi, M., 2020. Biosensors 10(9), 110.
Ferraz, M., Rho, H.S., Hemerich, D., Henning, H.H.W., van Tol, H.T.A., Hölker, M., Besenfelder, U., Mokry, M., Vos, P., Stout, T.A.E., Le Gac, S., Gadella, B.M., 2018. Nat Commun 9(1), 4934.
Ferrell, N., Desai, R.R., Fleischman, A.J., Roy, S., Humes, H.D., Fissell, W.H., 2010. Biotechnology and bioengineering 107(4), 707-716.
Fujimoto, T., Nakagawa, S., Morofuji, Y., Watanabe, D., Ujifuku, K., Horie, N., Izumo, T., Niwa, M., Banks, W.A., Deli, M.A., 2020. Cellular and molecular neurobiology 40(1), 113-121.
Gawad, S., Schild, L., Renaud, P., 2001. Lab on a Chip 1(1), 76-82.
Gerasimenko, T., Nikulin, S., Zakharova, G., Poloznikov, A., Petrov, V., Baranova, A., Tonevitsky, A., 2020. Frontiers in bioengineering and biotechnology 7, 474.
Giaever, I., Keese, C.R., 1991. Proceedings of the National Academy of Sciences 88(17), 7896-7900.
Gopinath, S.C., Lakshmipriya, T., Arshad, M.M., Uda, M., Al-Douri, Y., 2019. Nanoelectronics in biosensing applications. Nanobiosensors for Biomolecular Targeting, pp. 211-224. Elsevier.
Griep, L.M., Wolbers, F., de Wagenaar, B., ter Braak, P.M., Weksler, B., Romero, I.A., Couraud, P.O., Vermes, I., van der Meer, A.D., van den Berg, A., 2013a. Biomed. Microdevices 15(1), 145-150.
Griep, L.M., Wolbers, F., de Wagenaar, B., ter Braak, P.M., Weksler, B.B., Romero, I.A., Couraud, P.O., Vermes, I., van der Meer, A.D., van den Berg, A., 2013b. Biomed. Microdevices 15(1), 145-150.
He, Z., Zhang, Y., Feng, N., 2020. Materials Science and Engineering: C 106, 110298.
Henry, O.Y., Villenave, R., Cronce, M.J., Leineweber, W.D., Benz, M.A., Ingber, D.E., 2017a. Lab on a Chip 17(13), 2264-2271.
Henry, O.Y.F., Villenave, R., Cronce, M.J., Leineweber, W.D., Benz, M.A., Ingber, D.E., 2017b. Lab Chip 17(13), 2264-2271.
Hildebrandt, C., Büth, H., Cho, S., Thielecke, H., 2010. J. Biotechnol. 148(1), 83-90.
Hildebrandt, C., Thielecke, H., 2009. World Congress on Medical Physics and Biomedical Engineering, September 7-12, 2009, Munich, Germany, pp. 81-84. Springer.
Janvier, A., Kuo, C.-F., 2021. Summer Undergraduate Research Fellowship.
Jia, J., Wang, Z., Yue, T., Su, G., Teng, C., Yan, B., 2020. Chem. Res. Toxicol. 33(5), 1055-1060.
K'Owino, I.O., Sadik, O.A., 2005. Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis 17(23), 2101-2113.
Kalvøy, H., Frich, L., Grimnes, S., Martinsen, Ø.G., Hol, P.K., Stubhaug, A., 2009. Physiol. Meas. 30(2), 129.
Karanassios, V., 2018. InTech Publishing 1, 1-34.
Kennedy, C.C., Brown, E.E., Abutaleb, N.O., Truskey, G.A., 2021. Frontiers in cardiovascular medicine 8, 28.
Kerner, T.E., Paulsen, K.D., Hartov, A., Soho, S.K., Poplack, S.P., 2002. IEEE Trans. Med. Imaging 21(6), 638-645.
Kontturi, K., Murtomäki, L., Hirvonen, J., Paronen, P., Urtti, A., 1993. Pharm. Res. 10(3), 381-385.

Kozhevnikov, E., Hou, X., Qiao, S., Zhao, Y., Li, C., Tian, W., 2016. Journal of Materials Chemistry B 4(16), 2757-2767.
Lau, A.W.Y., Tan, L.T.-H., Ab Mutalib, N.-S., Wong, S.H., Letchumanan, V., Lee, L.-H., 2021. Progress In Microbes & Molecular Biology 4(1).
Lee, C.S., Leong, K.W., 2020. Curr. Opin. Biotechnol. 66, 78-87.
Lee, J., Kim, K., Kim, S., 2018. Methods Cell Biol. 146, 85-104.
Leung, C.M., De Haan, P., Ronaldson-Bouchard, K., Kim, G.-A., Ko, J., Rho, H.S., Chen, Z., Habibovic, P., Jeon, N.L., Takayama, S., 2022. Nature Reviews Methods Primers 2(1), 1-29.
Lewis, D.M., Mavrogiannis, N., Gagnon, Z., Gerecht, S., 2018. Biomicrofluidics 12(4), 042202.
Liang, Y., Yoon, J.-Y., 2021. Sensors and Actuators Reports 3, 100031.
Liu, K.Y., Nakatsu, C.H., Jones-Hall, Y., Kozik, A., Jiang, Q., 2021. Free Radical Biology and Medicine 163, 180-189.
Liu, L., Liu, X., 2019. Drug Transporters in Drug Disposition, Effects and Toxicity, 467-504.
Lo, C.-M., Keese, C.R., Giaever, I., 1995. Biophys. J. 69(6), 2800-2807.
Lu, Y., Chen, L., Li, L., Cao, Y., Journal of BioMed research international 2020.
Maass, C., Sorensen, N.B., Himmelfarb, J., Kelly, E.J., Stokes, C.L., Cirit, M., 2019. CPT: pharmacometrics & systems pharmacology 8(5), 316-325.
Majima, A., Handa, O., Naito, Y., Suyama, Y., Onozawa, Y., Higashimura, Y., Mizushima, K., Morita, M., Uehara, Y., Horie, H., 2017. J. Dig. Dis. 18(3), 151-159.
Maoz, B.M., Herland, A., Henry, O.Y., Leineweber, W.D., Yadid, M., Doyle, J., Mannix, R., Kujala, V.J., FitzGerald, E.A., Parker, K.K., 2017. Lab on a Chip 17(13), 2294-2302.
Marrero, D., Pujol-Vila, F., Vera, D., Gabriel, G., Illa, X., Elizalde-Torrent, A., Alvarez, M., Villa, R., 2021. Biosensors and Bioelectronics, 113156.
McCullough, S.D., 2020.
Mcelroy, M., Punton, J., Stephen, R., Wardrop, E., Maclellan, K., Tilley, L., Marsden, A., Baily, J., Milne, A., 2021. A Comparison of Alveolar-Capillary Barrier Damage and Inflammation in Two Short Term Rat Models of Acute Respiratory Distress Syndrome. TP127. TP127 Lung injury and mechanical ventilation, pp. A4663-A4663. American Thoracic Society.
Meghani, N., Kim, K.H., Kim, S.H., Lee, S.H., Choi, K.H., 2020. Archives of Pharmacal Research 43, 503-513.
Meijers, B., Farré, R., Dejongh, S., Vicario, M., Evenepoel, P., 2018. Toxins (Basel) 10(7), 298.
Mencattini, A., Mattei, F., Schiavoni, G., Gerardino, A., Businaro, L., Di Natale, C., Martinelli, E., 2019. Front. Pharmacol. 10, 100.
Meter, E.V.O., Manual, S.I., 2000. Inc. Sarasota, Fl, USA.
Millicell.
Miyazaki, T., Yang, J., Imamura, S., Hirai, Y., Kamei, K.-i., Tsuchiya, T., Tabata, O., 2021. 2021 IEEE 34th International Conference on Micro Electro Mechanical Systems (MEMS), pp. 411-414. IEEE.
Muendoerfer, M., Schaefer, U.F., Koenig, P., Walk, J.S., Loos, P., Balbach, S., Eichinger, T., Lehr, C.M., 2010. Int J Pharm 392(1-2), 134-140.
Nordberg, R.C., Zhang, J., Griffith, E.H., Frank, M.W., Starly, B., Loboa, E.G., 2017. Stem cells translational medicine 6(2), 502-511.
Nthunya, L.N., Gutierrez, L., Derese, S., Nxumalo, E.N., Verliefde, A.R., Mamba, B.B., Mhlanga, S.D., 2019. J. Chem. Technol. Biotechnol. 94(9), 2757-2771.
Oddo, A., Peng, B., Tong, Z., Wei, Y., Tong, W.Y., Thissen, H., Voelcker, N.H., 2019. Trends Biotechnol. 37(12), 1295-1314.
Otani, T., Furuse, M., 2020. Trends Cell Biol. 30(10), 805-817.
Pallarola, D., Bochen, A., Guglielmotti, V., Oswald, T.A., Kessler, H., Spatz, J.P., 2017. Anal. Chem. 89(18), 10054-10062.

Pasman, T., Baptista, D., van Riet, S., Truckenmüller, R.K., Hiemstra, P.S., Rottier, R.J., Stamatialis, D., Poot, A.A., 2020. Membranes 10(11), 330.
Patabendige, A., Skinner, R.A., Abbott, N.J., 2013. Brain Res. 1521, 1-15.
Pell, T.J., Gray, M.B., Hopkins, S.J., Kasprowicz, R., Porter, J.D., Reeves, T., Rowan, W.C., Singh, K., Tvermosegaard, K.B., Yaqub, N., 2021. SLAS DISCOVERY: Advancing the Science of Drug Discovery, 24725552211013077.
Peng, B., Tong, Z., Tong, W.Y., Pasic, P.J., Oddo, A., Dai, Y., Luo, M., Frescene, J., Welch, N.G., Easton, C.D., 2020. ACS Applied Materials & Interfaces 12(51), 56753-56766.
Pérez, P., Maldonado-Jacobi, A., López, A.J., Martínez, C., Olmo, A., Huertas, G., Yúfera, A., 2017. New Insights into Cell Culture Technology; InTech: Rijeka, Croatia, 135-155.
Pliquett, U., Prausnitz, M.R., 2000. Electrical impedance spectroscopy for rapid and noninvasive analysis of skin electroporation. Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery, pp. 377-406. Springer.
Poenar, D.P., Yang, G., Wan, W.K., Feng, S., 2020. Materials (Basel) 13(10).
Polacheck, W.J., Kutys, M.L., Tefft, J.B., Chen, C.S., 2019. Nat. Protoc. 14(5), 1425-1454.
Pradhan-Sundd, T., Monga, S.P., 2019. Gene Expression 19(2), 69.
Pranzo, D., Larizza, P., Filippini, D., Percoco, G., 2018. Micromachines 9(8), 374.
Quirós-Solano, W., Gaio, N., Stassen, O., Arik, Y., Silvestri, C., Van Engeland, N., Van der Meer, A., Passier, R., Sahlgren, C., Bouten, C., 2018. Sci. Rep. 8(1), 1-11.
Rahim, S., Üren, A., 2011. Journal of visualized experiments: JoVE(50).
Raimondi, I., Izzo, L., Tunesi, M., Comar, M., Albani, D., Giordano, C., 2020. Frontiers in bioengineering and biotechnology 7, 435.
Reichl, S., 2008. J Pharm Pharmacol 60(3), 299-307.
Risueño, I., Valencia, L., Jorcano, J., Velasco, D., 2021. APL bioengineering 5(3), 030901.
Robinson, J.M., Abey, S.A., Kenea, N.D., Henderson, W.A., 2018. bioRxiv, 355552.
Sakolish, C.M., Esch, M.B., Hickman, J.J., Shuler, M.L., Mahler, G.J., 2016a. EBioMedicine 5, 30-39.
Sakolish, C.M., Esch, M.B., Hickman, J.J., Shuler, M.L., Mahler, G.J., 2016b. EBioMedicine 5, 30-39.
Salih, A.R.C., Farooqi, H.M.U., Kim, Y.S., Lee, S.H., Choi, K.H., 2020. Microelectron. Eng. 232, 111405.
Salminen, A., Allahyari, Z., Gholizadeh, S., McCloskey, M., Ajalik, R., Cottle, R., Gaborski, T., McGrath, J., 2020. vitro Studies of Transendothelial Migration for Biological and Drug Discovery.
Santa-Maria, A.R., Walter, F.R., Figueiredo, R., Kincses, A., Vigh, J.P., Heymans, M., Culot, M., Winter, P., Gosselet, F., Dér, A., 2021. J. Cereb. Blood Flow Metab., 0271678X21992638.
Santbergen, M.J., van Der Zande, M., Bouwmeester, H., Nielen, M.W., 2019. TrAC, Trends Anal. Chem. 115, 138-146.
Sarac, E., Meiwes, A., Eigentler, T., Forchhammer, S., Kofler, L., Häfner, H.-M., Garbe, C., 2020. Acta Derm. Venereol.
Sarmento, B., Andrade, F., da Silva, S.B., Rodrigues, F., das Neves, J., Ferreira, D., 2012. Expert Opin. Drug Metab. Toxicol. 8(5), 607-621.
Schneider, S., Gruner, D., Richter, A., Loskill, P., 2021. Lab on a Chip.
Shrestha, J., Razavi Bazaz, S., Aboulkheyr Es, H., Yaghobian Azari, D., Thierry, B., Ebrahimi Warkiani, M., Ghadiri, M., 2020. Crit. Rev. Biotechnol. 40(2), 213-230.
Soley, A., Lecina, M., Gámez, X., Cairo, J., Riu, P., Rosell, X., Bragos, R., Godia, F., 2005. J. Biotechnol. 118(4), 398-405.
Soucy, J.R., Bindas, A.J., Koppes, A.N., Koppes, R.A., 2019. Iscience 21, 521-548.
Soum, V., Kim, Y., Park, S., Chuong, M., Ryu, S.R., Lee, S.H., Tanev, G., Madsen, J., Kwon, O.-S., Shin, K., 2019. Micromachines 10(2), 109.
Spadoni, I., Fornasa, G., Rescigno, M., 2017. Nature Reviews Immunology 17(12), 761-773.

Srinivasan, B., Kolli, A.R., 2019. Transepithelial/Transendothelial Electrical Resistance (TEER) to Measure the Integrity of Blood-Brain Barrier. Blood-Brain Barrier, pp. 99-114. Springer.
Srinivasan, B., Kolli, A.R., Esch, M.B., Abaci, H.E., Shuler, M.L., Hickman, J.J., 2015a. Journal of laboratory automation 20(2), 107-126.
Srinivasan, B., Kolli, A.R., Esch, M.B., Abaci, H.E., Shuler, M.L., Hickman, J.J., 2015b. Journal of laboratory automation 20(2), 107-126.
Strand-Amundsen, R., Tronstad, C., Kalvøy, H., Gundersen, Y., Krohn, C., Aasen, A., Holhjem, L., Reims, H., Martinsen, Ø.G., Høgetveit, J., 2016. Physiol. Meas. 37(2), 257.
Strand-Amundsen, R.J., Tronstad, C., Kalvøy, H., Ruud, T.E., Høgetveit, J.O., Martinsen, Ø.G., Tønnessen, T.I., 2018. Physiol. Meas. 39(2), 025001.
Sun, T., Morgan, H., 2010. Microfluid. Nanofluid. 8(4), 423-443.
Sutterby, E., Thurgood, P., Baratchi, S., Khoshmanesh, K., Pirogova, E., 2020. Small, 2002515.
Szulcek, R., Bogaard, H.J., van Nieuw Amerongen, G.P., 2014. Journal of visualized experiments: JoVE(85).
Thomson, A., Smart, K., Somerville, M.S., Lauder, S.N., Appanna, G., Horwood, J., Raj, L.S., Srivastava, B., Durai, D., Scurr, M.J., 2019. BMC Gastroenterol. 19(1), 1-14.
Tsukita, S., Furuse, M., Itoh, M., 2001. Nature reviews Molecular cell biology 2(4), 285-293.
Tu, K.-H., Yu, L.-S., Sie, Z.-H., Hsu, H.-Y., Al-Jamal, K.T., Wang, J.T.-W., Chiang, Y.-Y., 2021. Micromachines 12(1), 37.
van der Helm, M.W., Henry, O.Y., Bein, A., Hamkins-Indik, T., Cronce, M.J., Leineweber, W.D., Odijk, M., van der Meer, A.D., Eijkel, J.C., Ingber, D.E., 2019a. Lab on a Chip 19(3), 452-463.
van der Helm, M.W., Henry, O.Y.F., Bein, A., Hamkins-Indik, T., Cronce, M.J., Leineweber, W.D., Odijk, M., van der Meer, A.D., Eijkel, J.C.T., Ingber, D.E., van den Berg, A., Segerink, L.I., 2019b. Lab Chip 19(3), 452-463.
van der Helm, M.W., Odijk, M., Frimat, J.-P., van der Meer, A.D., Eijkel, J.C., van den Berg, A., Segerink, L.I., 2016. Biosensors and bioelectronics 85, 924-929.
Van Meenen, J., Ní Dhubhghaill, S., Van den Bogerd, B., Koppen, C., 2021. Tissue Engineering Part B: Reviews.
Vancamelbeke, M., Vermeire, S., 2017. Journal of Expert review of gastroenterology & hepatology 11(9), 821-834.
Vogel, P.A., Halpin, S.T., Martin, R.S., Spence, D.M., 2011. Anal. Chem. 83(11), 4296-4301.
Wanat, K., 2020. Mol. Biol. Rep. 47(4), 3221-3231.
Wang, L., Zhang, F., Lu, K., Abdulaziz, M., Li, C., Zhang, C., Chen, J., Li, Y., 2020a. Journal of nanobiotechnology 18, 1-15.
Wang, X., Hou, Y., Ai, X., Sun, J., Xu, B., Meng, X., Zhang, Y., Zhang, S., 2020b. Biomed. Pharmacother. 132, 110822.
Wegener, J., Seebach, J., 2014. Cell Tissue Res 355(3), 485-514.
World Precision Instruments.
Wu, Q., Liu, J., Wang, X., Feng, L., Wu, J., Zhu, X., Wen, W., Gong, X., 2020a. Biomed. Eng. Online 19(1), 1-19.
Wu, S., Wang, X., Li, Z., Zhang, S., Xing, F., 2020b. Micromachines 11(12), 1059.
Wufuer, M., Lee, G., Hur, W., Jeon, B., Kim, B.J., Choi, T.H., Lee, S., 2016. Sci. Rep. 6, 37471.
Xiao, Y., Tang, Z., Wang, J., Liu, C., Kong, N., Farokhzad, O.C., Tao, W., 2020. Angew. Chem. Int. Ed. 59(45), 19787-19795.
Xu, Y., Xie, X., Duan, Y., Wang, L., Cheng, Z., Cheng, J., 2016. Biosensors and Bioelectronics 77, 824-836.
Yan, L., 2020. Boston University Theses & Dissertations.
Yeste, J., García-Ramírez, M., Illa, X., Guimerà, A., Hernández, C., Simó, R., Villa, R., 2018. Lab on a Chip 18(1), 95-105.
Yeste, J., Illa, X., Gutiérrez, C., Solé, M., Guimerà, A., Villa, R., 2016. J. Phys. D: Appl. Phys. 49(37), 375401.

Yu, X., Ji, C., Shao, A., 2020. Front. Neurosci. 14, 334.
Yu, Z., Hao, R., Zhang, Y., Yang, H., 2021. 2021 IEEE 34th International Conference on Micro Electro Mechanical Systems (MEMS), pp. 982-985. IEEE.
Yuste, Y., Serrano, J.A., Olmo Fernández, A., Maldonado-Jacobi, A., Pérez García, P., Huertas Sánchez, G., Pereira, S., Portilla, F.d.l., Yúfera García, A., 2018. BIODEVICES 2018: 11th International Joint Conference on Biomedical Engineering Systems and Technologies (2018), p 96-99. ScitePress Digital Library.
Zanetti, F., 2020. Kidney-on-a-chip. Organ-on-a-chip, pp. 233-253. Elsevier.
Zhang, Q., Sito, L., Mao, M., He, J., Zhang, Y.S., Zhao, X., 2018.
Zhao, Y., Rafatian, N., Wang, E.Y., Wu, Q., Lai, B.F., Lu, R.X., Savoji, H., Radisic, M., 2020. Adv. Drug Del. Rev. 165, 60-76.
Zhu, Y., Mandal, K., Hernandez, A.L., Kawakita, S., Huang, W., Bandaru, P., Ahadian, S., Kim, H.-J., Jucaud, V., Dokmeci, M.R., 2021. Current Opinion in Biomedical Engineering, 100309.

Highlights

- Report on state-of-the-art advances in TEER measurement in biological barriers-on-chips (organ-on-chip)
- Review of latest applications of advanced techniques and strategies for increasing the quality of TEER measurements.
- Highlight challenges and future trends in developing next-generation TEER securement systems using sensitive electrodes, microfluidic devices, stem cell technology, and membrane design.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: