From impedance to TEER

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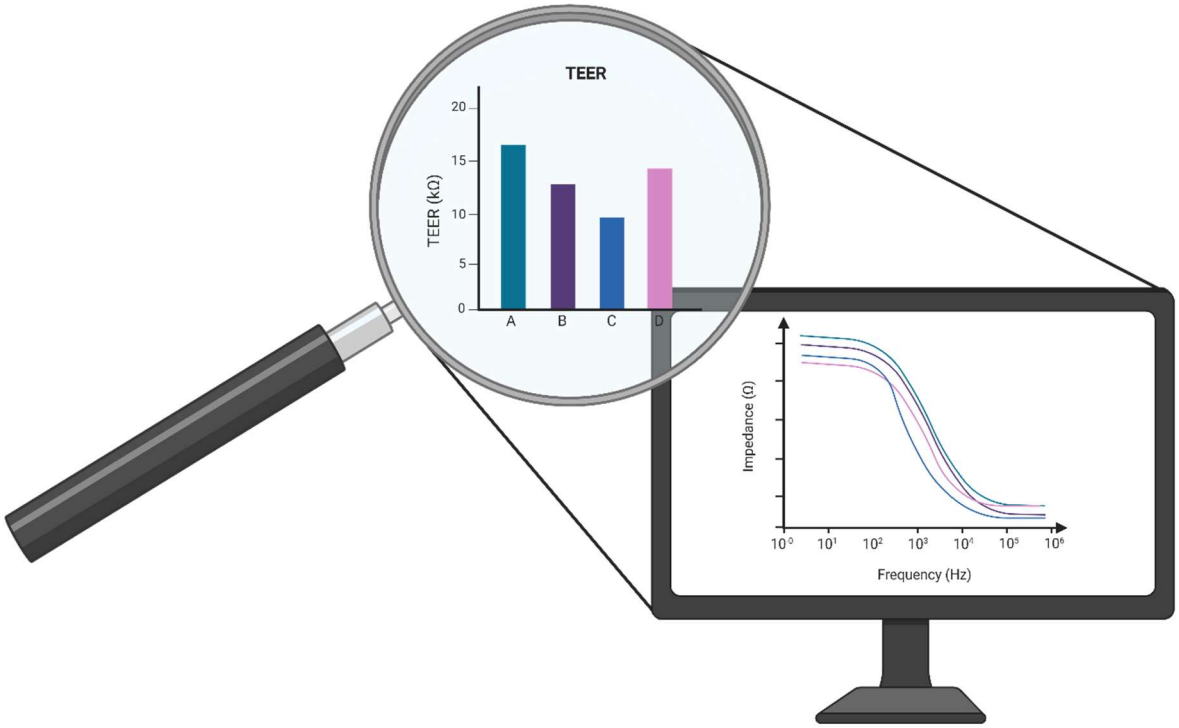

Introduction

Numerous tissue types have a barrier function to protect the body from xenobiotic compounds, e.g. the gut [1], the lungs [2], the blood-brain barrier [3], the skin [4] etc. Impairment of this function, caused by several possible diseases can lead to serious complications. Therefore, barrier function analysis is important for toxicology research, drug discovery, and disease understanding. Conventional barrier function analysis is performed via end-point measurement e.g. immunohistochemistry staining or freeze-fracture electron microscopy, which led to the need for non-invasive continuous measuring techniques. Transendothelial/transepithelial electrical resistance (TEER) detection is such a non-invasive technique which allows the user to measure the tight junction resistance of a tissue culture [5].

Through the application of an electrical current, the resistance of the tight junctions, is determined in Ω or Ω*cm^2. Conventional TEER devices use a single frequency to measure the TEER, typically 12.5 Hz. While this gives a good indication of the true TEER, other factors that influence the resistance are unjustly neglected. Furthermore, the positioning of the electrodes, which are in most cases handheld, influences the results, thereby further jeopardizing the reliability and reproducibility [6].

Another method to measure the TEER is to measure the complete impedance across a cell layer over a wide range of frequencies; full spectrum impedance spectroscopy. This ensures a more detailed understanding of all factors that contribute to the total resistance/impedance [5], [6]. A

challenge using full spectrum impedance analysis is to correctly extract the TEER from these results. In this paper we discuss equivalent circuit model (ECM) fitting and how to extract TEER from full spectrum impedance measurements.

Principal of equivalent circuit model (ECM) fitting

When impedance is measured an alternating current (AC) is sent through the electrodes, medium, and cell layer. This current flows from electrode 1 to electrode 2 and back, at different frequencies. While doing this, it encounters several "obstacles" on its path, such as the electrode-medium interface, the medium itself, the cell layer etc. All these obstacles together contribute to the total impedance and can be visualized as an electrical circuit, where these obstacles are visualized as elements in the electrical circuit. With ECM fitting, the goal is to select a circuit that best mimics the path that the electrical current follows.

Equivalent circuits

To select equivalent circuits, we have to find a circuit element for each obstacle in the cell culture. Generally biological cell cultures such as Transwell cultured cell layers consist of two types of elements: resistors and capacitors [5], [7], [8]. However, the electrodes and wiring of the detector may also introduce inductors and constant phase elements (CPEs), to the equivalent circuit. Here is a short explanation of each element and figure of all icons visualizing the elements [9]:

Resistor (R): A resistor impedes the alternating current and therefore increases the total impedance. A resistor will always impede the current by the same amount, measured in Ohm (Ω) and is frequency independent [10]. A resistor is visualized as an empty rectangle, see **Figure 1**.

Capacitor (C): A capacitor changes the alternating current by shifting the phase, making the voltage lag behind. The size of the phase shift is dependent on the strength, measured in (micro)Fahrad ((μ)F), of the capacitor and the frequency of the applied AC current. At higher frequencies the size of the phase shift decreases, therefore the capacitor affects the signal less at higher frequencies [10]. A capacitor consists of 2 vertical lines, see **Figure 1**.

Inductor (L): An inductor changes the alternating current by phase shifting the voltage forward. The size of the phase shift is dependent on the strength of the inductor and the frequency. At higher frequencies the size of the phase shift increases as the inductor affects the signal more at higher frequencies. The inductive effects in the circuit are not caused by anything in the Transwell, but might be present in the equivalent circuit due to wiring between the hardware and the electrodes. It is therefore possible an inductor, however with a small strength measured in (micro)Fahrad ((μ)F), is still a part of the system of which the impedance is measured [10]. An inductor is visualized as three semi-circles, see **Figure 1**.

Constant Phase Element (CPE): A constant phase element is an equivalent circuit fitting element. Its purpose is to mimic the behavior of the imperfect capacitor. An imperfect capacitor is created by the double layer at the interface of an electrode and medium. A CPE is defined by two parameters, the admittance, and the phasance. The admittance determines how strong the magnitude of the impedance of the constant phase element changes with frequency, where higher impedance means weaker change in magnitude of the impedance. The phasance determines the phase shift. This phase shift is a value between 0 and 1, where 0 means the CPE mimics a perfect resistor and 1 means the CPE mimics a perfect capacitor. Because of this, the

phase is independent of frequency, which is why it is called a constant phase element [9]. A CPE is indicated with the head of two arrow pointing to the left, see **Figure 1**.

Warburg element: A Warburg element is a constant phase element with the value of the phasance set to ½. A Warburg element is visualized as a "W", see **Figure 1**.

RC-circuit: It is possible that an element is not either a resistor or a capacitor, but both, e.g. a resistor (R) and capacitor (C) in parallel [11], **see Figure 1**. When this happens, the current can choose to either go through the resistor or the capacitor, this is called an RC circuit. Because of the capacitor, the impedance of the element is frequency dependent. At high frequencies, the capacitor becomes almost fully conductive, therefore the current will choose the capacitor path and the resistors effect is negligible. At low frequencies the capacitor becomes very resistive and the current mainly travels through the resistance, leading to the RC-parallel circuit to behave like it is only the resistor.

RCPE-circuit: Instead of RC-circuit where a resistor and a capacitor are in parallel, an RPCE circuit is a parallel circuit or a resistor (R) and a constant phase element (CPE) see **Figure 1** [9]. It behaves similarly to a RC-circuit, except for the phase shift, which is less pronounced [12].

Randles circuit: A Randles circuit is an equivalent circuit made to model the behavior of an electrode-liquid interface. There are several layouts possible, but all layouts contain a resistance, capacitor and a Warburg element. The most common type has a resistor in series with a Warburg element, all in parallel with a capacitor see **Figure 1**. [13]

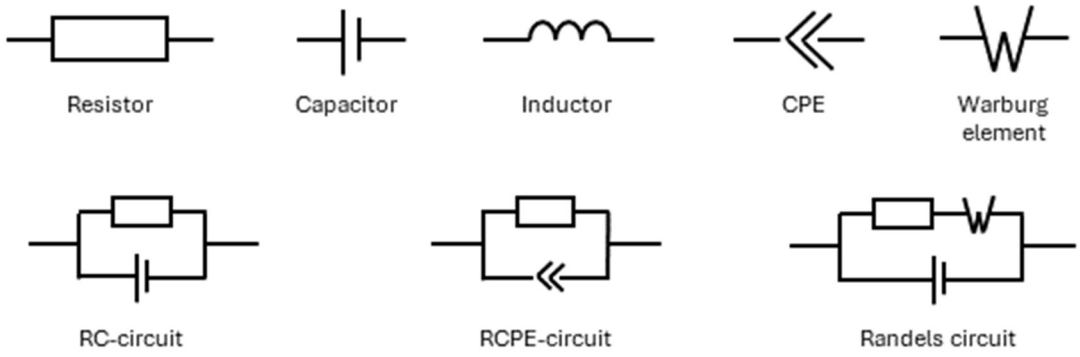

Figure 1. Icon of all discussed elements and visualization of the discussed parallel circuits. *A resistor is a rectangle, a capacitor is a combination of a long and short vertical line, an inductor a concatenation of three semi-circles, a CPE is a combination of two arrowheads pointing to the left, and a Warburg element a "W". An RC-circuit is a parallel circuit of a resistor and a capacitor, An RCPE-circuit is a parallel circuit of a resistor and a CPE, and a Randels circuit is a resistor and Warburg element in parallel with a capacitor*

Now we know which electrical elements we can choose, we need to find all the "obstacles" of our cell culture and assign an electrical element to them. A visual representation of the cell culture and the electrical circuit is depicted in **Figure 2**.

Let's start with the electrical wiring to the electrode. As previously discussed, none of the components in the Transwell function as inductors; however, the wiring might have inductive properties depending on its length. The effect of wiring is oppositional to the effect of a capacitor, thereby acting like a conductor. It is not necessarily part of the equivalent circuit, but it might improve the accuracy, while not increasing the complexity too much.

The electrodes are a little harder to assign an element to. Literature describes three main choices: an RC-circuit [11], an RCPE-circuit [9], or a Randles circuit. To simplify circuit fitting it

might be best to fit the perhaps less accurate, but easier to fit, RC-parallel circuit. Another option is to fit the RCPE-parallel circuit but set the constant phase element to have a fixed constant phase. The reason to use an RCPE-circuit is because the capacitance caused by the double layer between the electrode and the medium is not a perfect capacitor and behaves somewhere between a capacitor and resistor. A CPE is designed to simulate this behavior. A Randles circuit is used when there is the possibility of a faradic reaction at the electrode interface, when this happens a straight line can be found in the Nyquist plot at low frequency, which is equivalent to a phase that is tending towards -45º in the corresponding Bode plot/frequency versus phase plot.

The medium behaves like a decent conductor, so we can simply fit a resistor as its equivalent circuit element [5], [7], [8].

Then, the element for the cell culture of course. The behavior of the cell culture looks a lot like that of an RC-parallel circuit. The theoretical explanation for this is [5], [7], [8] that the current can either go through the cell itself, where the cell membrane behaves like a capacitor, or it can pass between the cells and go through the tight junctions, which behave like resistors. This resistive element corresponds to the TEER. Some cell types have more of these layers of cells on top of each other e.g. a skin construct, which ensures multiple RC-circuits. The sum of all the resistors is the total TEER.

The Transwell membrane which contains the cells is another resistor [5], [7], [8].

The other electrode behaves similar to the first electrode, meaning this can be visualized as an RC-circuit or an RCPE-circuit.

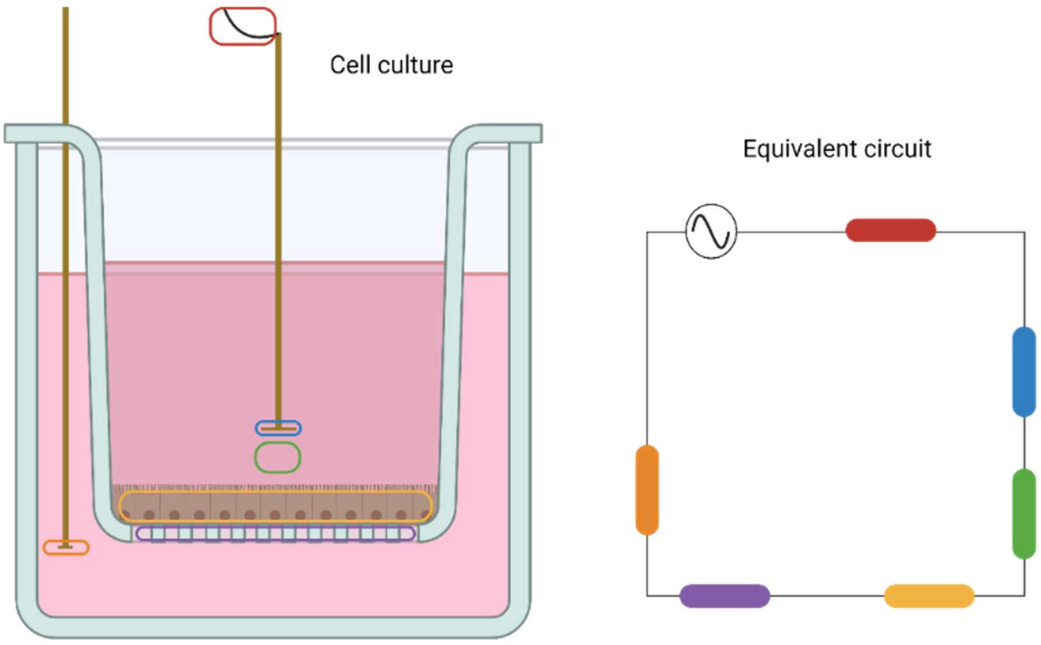

Figure 2. Visual representation of equivalent circuit model fitting. *On the left a representative image of a transwell containing a cell layer, visualizing all the "obstacle" and on the right an electrical circuit visualizing all the obstacles from the transwell as a circuit elements.*

Choosing a model

The above-mentioned circuit can be the base for the development for a fitting circuit, but since all cells, cell layers, and Transwells are different, a specific circuit has to be found for each cell culture. When a circuit is simulated, the theoretical impedance of this simulated circuit can be calculated and compared to the observed impedance data. If the theoretical impedance data is exactly the same as the observed impedance data, the circuit perfectly mimics the cell culture, however in practice there will be slight deviations. It is much more likely there are some differences between the theoretical impedance and the measured impedance. We call the combination of these differences the error of the circuit. The equivalent circuit with the smallest error, is the best fitting circuit.

One way to determine the error between the model and observed data is by using the distance between the theoretical impedance and the observed impedance in the analytic representation. As discussed before, elements can increase the magnitude of the impedance (e.g. resistors), but also change the phase (e.g. capacitors). To represent both the magnitude and phase, complex values are used: $Z = R + jX$ where the real part is called the resistance, R, and the imaginary part is called the reactance $X[14]$. The complete mathematics of imaginary and complex numbers is beyond the scope of this white paper. However, important to understand is that a complex number can be visualized as a point in a 2-dimensional space. Here the coordinate on the x-axis the is the resistance, R, and the coordinate on the y-axis is the Reactance, X, as visualized in **Figure 3**. The magnitude, $|\tilde{Z}|$, of the impedance is the length of the arrow, and the phase, θ, of the impedance is the angle between the arrow and the x-axis . When knowing the magnitude of the arrow and the phase angle, the value of the real part and the imaginary part can be calculated using standard trigonometric functions. The real part is $R = |\tilde{Z}| \cdot \cos(\theta)$ and the imaginary part is $X = |\tilde{Z}| \cdot \sin(\theta)$.

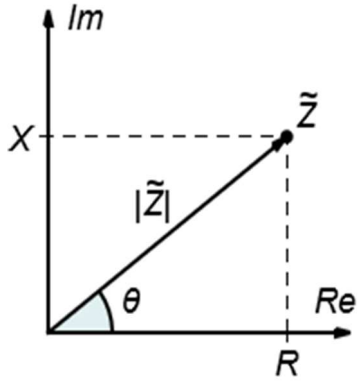

Figure 3: visualization of a complex point. *A complex point represented as both R+jX and as a magnitude (|Z|) with phase (θ)*

Another reason for using the complex representation is that it is easier to distinguish certain circuit elements. Resistors only influence the real part of the impedance, while capacitors (negatively) and inductors (positively) influence the imaginary part of the impedance. The equivalent circuits generally consist of these three components so to have a good way to separate them is rather convenient [10].

Mathematics

When the best theoretical equivalent circuit is chosen, all the values of each element or obstacle can be calculated. Since the TEER is one of the elements the TEER can be extracted, from the theoretical equivalent circuit. The other way around, when you know all the values of the elements, the total impedance can be calculated.

In this section we explain the mathematics of total impedance from different circuit models. Important to know: elements in series add to each other: $Z_{tot} = Z_1 + Z_2$ and elements in parallel add as follows: $\frac{1}{Z_{tot}} = \frac{1}{Z_1} + \frac{1}{Z_2}$ [10].

Below in **Figure 4** you find two different circuits. The left circuit consists of two resistors in series, the right circuit consists of a resistor and a capacitor in parallel. With the rules above we can calculate the value of the total impedance.

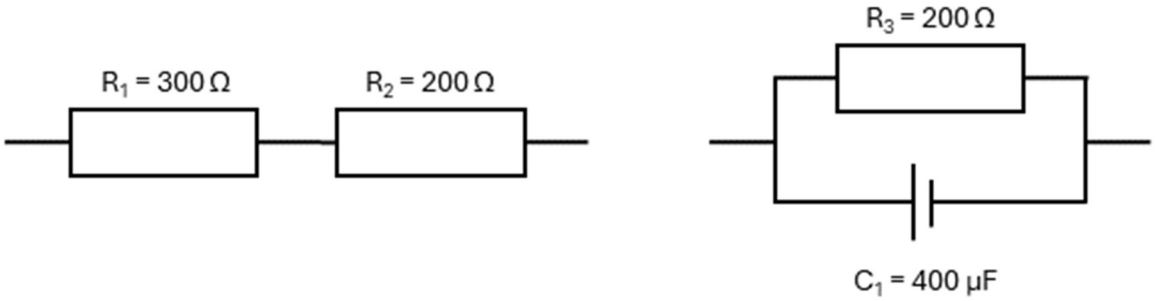

***Figure 4. Example circuits**. On the left two resistors in series and on the right a resistor in parallel to a capacitor, also known as a RC-parallel circuit.*

We can calculate the impedance of the left circuit by simply adding the individual impedances together:

$$Z_{tot} = R_1 + R_2 = 300 + 200 = 500\ \Omega.$$

The second circuit is a little more difficult for several reasons. First, the impedance caused by the capacitor is imaginary and is dependent on the input frequency, which we will call this ω the angular frequency. $\omega = 2\pi f$, with f being the frequency. The impedance of a capacitor is given by $Z_C(\omega) = \frac{1}{C\omega j}$, with j being the imaginary unit. Secondly, the elements being in parallel also further increases the complexity. Putting all of this together we get:

$$\frac{1}{Z_{tot}(\omega)} = \frac{1}{R_3} + \frac{1}{\frac{1}{C_1\omega j}} = \frac{1}{R_3} + C_1\omega j = \frac{1 + R_3C_1\omega j}{R_3} \rightarrow$$

$$Z_{tot}(\omega) = \frac{R_3}{1 + R_3C_1\omega j} = \frac{R_3}{1 + R_3C_1\omega j} \cdot \frac{1 - R_3C_1\omega j}{1 - R_3C_1\omega j} = \frac{R_3}{1 + (R_3C_1\omega)^2} - \frac{R_3^2C_1\omega}{1 + (R_3C_1\omega)^2} j$$

$$Z_{tot}(\omega) = \frac{200}{1 + (200 \cdot 400 \cdot 10^{-6} \cdot \omega)^2} - \frac{200^2 \cdot 400 \cdot 10^{-6} \cdot \omega}{1 + (200 \cdot 400 \cdot 10^{-6} \cdot \omega)^2} j$$

$$= \frac{200}{1 + (0.08\omega)^2} - \frac{16\omega}{1 + (0.08\omega)^2} j$$

As you can see in this example, the two elements influence each other a lot more, despite the imaginary part of the impedance being caused by the capacitor, the resistance term R_3 can also be found in the Reactance, and the full impedance is now frequency dependent, despite only the capacitance being frequency dependent. We will not have to do this full calculation as the software is perfectly fine capable of calculating results with complex values, but it is good to have some understanding of the results of these equations, especially for such fundamental circuit elements as the RC-parallel circuit. Notice for example that when the frequency tends to 0 only the resistance has an influence, as the capacitor becomes near impassable. Also notice when the frequency tends towards infinity, the capacitor becomes fully conductive, and the impedance drops to zero.

$$\lim_{\omega \to 0} Z_{tot}(\omega) = \frac{200}{1 + (0.08 \cdot 0)^2} - \frac{16 \cdot 0}{1 + (0.08 \cdot 0)^2} j = 200$$

$$\lim_{\omega \to \infty} Z_{tot}(\omega) = \lim_{\omega \to \infty} \frac{200}{1 + (0.08 \cdot \omega)^2} - \frac{16 \cdot \omega}{1 + (0.08 \cdot \omega)^2} j \to 0$$

Summary

So, circuit fitting is an accurate manner to calculate TEER out of full spectrum frequency data. To fit the model, simply calculate the theoretical impedance for the given parameters at each measured frequency and compare the results with the observed results. Then figure out how to change the parameters to decrease the error between the observed data and theoretical results and change the parameters until the error is small enough, or it is no longer possible to find a better minimum. A representation of the workflow is visualized in **Figure 55**.

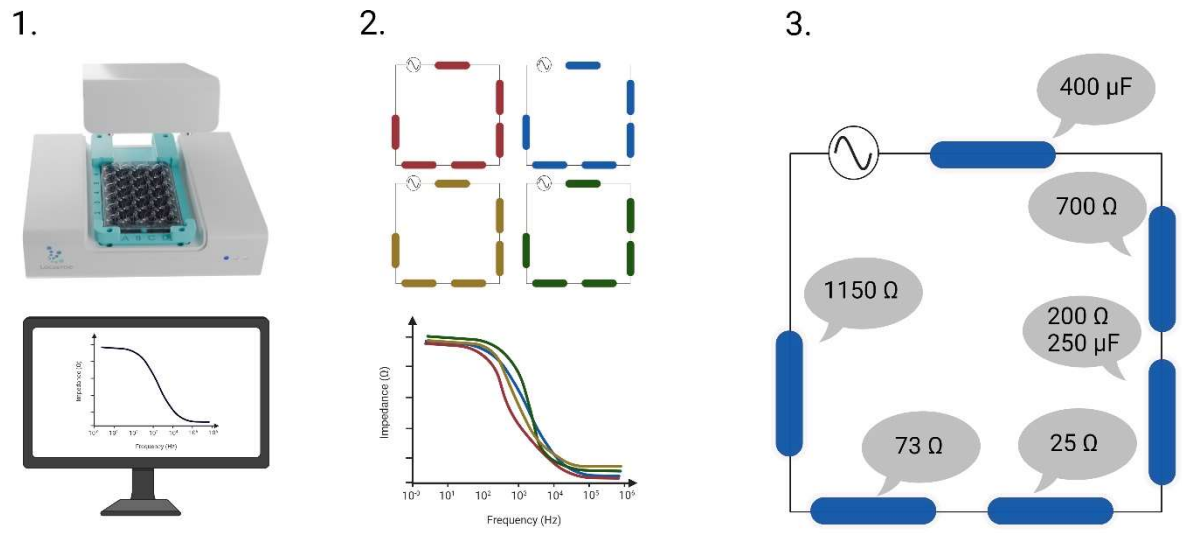

Figure 5. ECM fitting workflow. *Step 1, perform a measurement with the Artemis. Step 2, create technical models, fit the parameters for each model and choose the parameter set with the smallest error. Step 3, choose the model with the smallest error given the fitted parameter values and determine the TEER from the fitted parameters.*

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