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A simple approach to perform TEER measurement using a self-made volt-amperemeter with programmable output frequency

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TITLE:

A Simple Approach to Perform TEER Measurements Using a Self-Made Volt-Amperemeter with Programmable Output Frequency

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KEYWORDS:

transepithelial electrical resistance, TEER, 4 terminal sensing, barrier, voltohmmeter, voltammeter

SUMMARY:

Here, we demonstrate how to set up an inexpensive volt-amperemeter with programmable output frequency that can be used with commercially available chopstick electrodes for transepithelial/endothelial electrical resistance measurements.

ABSTRACT:

Transepithelial/endothelial electrical resistance (TEER) has been used since the 1980s to determine confluency and permeability of in vitro barrier model systems. In most cases, chopstick electrodes are used to determine the electric impedance between the upper and lower compartment of a cell culture filter insert system containing cellular monolayers. The filter membrane allows the cells to adhere, polarize, and interact by building tight junctions. This technique has been described with a variety of different cell lines (e.g., cells of the blood-brain barrier, blood-cerebrospinal fluid barrier, or gastrointestinal and pulmonary tract). TEER measurement devices can be readily obtained from different laboratory equipment suppliers. However, there are more cost-effective and customizable solutions imaginable if an appropriate voltammeter is self-assembled. The overall aim of this publication is to set up a

reliable device with programmable output frequency that can be used with commercially available chopstick electrodes for TEER measurement.

INTRODUCTION:

Epithelial and endothelial cells function as cellular boundaries, separating the apical and basolateral sides of the body. If they are connected through tight junctions, passive substance diffusion through the paracellular spaces is restricted[1], resulting in the formation of a selectively permeable barrier. Several artificial barrier systems have been developed[2] using microvascular endothelial cells (HBMEC, blood-brain barrier[3-7]), choroid plexus epithelial cells (HIBCPP/PCPEC, blood-cerebrospinal fluid barrier[8-14]), colorectal adenocarcinoma cells (Caco-2, gastrointestinal models[15]), or airway/alveolar cell lines (pulmonary models[16,17]). These systems typically consist of cells grown in a monolayer on permeable membranes (i.e., filter insert systems) to allow access to the apical and basolateral sides. It is important that the integrity of the model system matches the in vivo conditions. Hence, several techniques have been developed to analyze barrier function by measuring paracellular diffusion of tracer compounds across the cell layer. These substances include radiolabeled sucrose, dye-labeled albumin, FITC-labeled inulin, or dye-labeled dextrans[2]. However, chemical dyes can make cells unusable for further experiments. To monitor barrier systems noninvasively, measurement of transepithelial/transendothelial electrical resistance (TEER) across a cellular monolayer can be used[2,18,19]. Because bipolar electrode systems are influenced by the electrode polarization impedance at the electrode-electrolyte interface, tetrapolar measurements are generally used to overcome this limitation[20]. The underlaying technique is a four-terminal sensing (4T) that was first described in 1861 by William Thomson (Lord Kelvin)[21]. In brief, the current is injected by a pair of current-carrying electrodes while a second pair of voltage-sensing electrodes is used to measure the voltage drop[20]. Nowadays, so-called chopstick electrodes consist of a pair of double electrodes, each containing a silver/silver-chloride pellet for measuring voltage and a silver electrode for passing current[2]. The electrical impedance is measured between the apical and the basolateral compartment with the cell layer in between (**Figure 1**). A square wave signal at a frequency of typically 12.5 Hz is applied at the outer electrodes and the resulting alternating current (AC) measured. Additionally, the potential drop across the cell layer is measured by the second (inner) electrode pair. Electrical impedance is then calculated according to Ohm's law. TEER values are normalized by multiplying impedance and cell layer surface area and are typically expressed as $\Omega \cdot cm^2$.

There are systems in which cells and electrodes are arranged in a more sophisticated way, but are also based on the 4T measuring principle and can be used with the same measurement devices. EndOhm systems, for example, in which the filter is inserted, contain a chamber and cap with a pair of concentric electrodes with the same structure as the chopstick electrode. The shape of the electrodes allows for a more uniform current density flow across the membrane, thereby reducing variation between readings. Even more complex (but also more accurate) is an Ussing chamber, where a cell layer separates two chambers filled with Ringer's solution[22]. The chamber itself can be gassed with oxygen, CO_2, or N_2), and stirred or supplemented with experimental substances. As ion transport across the cell layer occurs, a potential difference can be measured

by two voltage-sensing electrodes near the tissue. This voltage is cancelled out by two current-carrying electrodes placed next to the cell layer. The measured current will then give the net ion transport and the transepithelial resistance, which reflects barrier integrity, can be determined[22]. TEER measurement can also be applied on body-on-a-chip systems that represent barrier-tissue models[23,24]. These systems mimic in vivo conditions of the cells and often consist of several types of cells, stacked on top of each other in layers.

The following protocol explains how to set up a cost-effective and reliable voltammeter with programmable output frequency that produces no statistically significant differences in TEER compared to commercially available measurement systems.

PROTOCOL:

1. Assembly of a basic volt-amperemeter for TEER measurement

1.1. Prepare a standard USB charger as the 5 V D.C. power supply, a USB extension cord, a microcontroller that will be used as a programmable square wave generator, two standard multimeters that are able to measure alternating current and voltage as root mean square (True-RMS), four cables with banana plugs, a telephone extension cord with a RJ14 female connector including six pins with the inner four wired (6P4C), two short cables, a luster terminal, a 120 kΩ pre-resistor, wire end ferrules, and soldering lugs. The tools required are an insulation stripper, a crimping tool, and a soldering iron.

1.2. First, connect the USB extension to the microcontroller board.

1.3. Strip the end insulation of two short cables. Solder one side per cable either directly to pins 0 and 2 of the microcontroller or to soldering lugs, which in turn are clipped on the respective pins. Crimp the other ends to the wire end ferrules and connect them to a luster terminal as depicted in **Figure 1**.

1.4. Link the banana plugs to the multimeters. Strip and crimp the other end of each of the four cables.

1.5. Cut the telephone extension cord in two pieces and dismantle and crimp the conductors of the side containing the female connector. Check for the continuity of the conductors and pins.

1.6. The first multimeter will be used to measure current in µA (note that the AC mode has to be set explicitly). Connect it in a series with a 120 kΩ pre-resistor to pins five and six of the RJ14 connector, corresponding to the outer electrode pair of the chopstick electrode.

1.7. Finally, link the second multimeter, which will be used to measure the transepithelial voltage drop in mV, via the luster terminal to pins three and four of the RJ14 connector, corresponding to the inner electrode pair of the chopstick electrode.

1.8. If desired, mount the installation in a chassis.

2. Programming the microcontroller

2.1. Modify the provided source code (supplemental coding file 1) as needed. In the given form, pins 0 and 2 will alternate between ground and +5 V with 40 ms half-time of oscillation. Thus, a square wave signal with an amplitude of 5 V and a frequency of approximately 12.5 Hz will be generated. The real values may differ due to the inaccuracy of the microcontroller's time emitter.

2.2. Connect the microcontroller to a desktop computer via a USB port and upload the source code with matching software[25].

3. Recording of voltage oscillograms (optional)

3.1. Bypass pins five and six of the RJ14 connector with a 1 kΩ test resistor and connect to an oscilloscope.

3.2. Check for the frequency, peak voltage, and waveform. Digitize and export the data.

3.3. If desired, record oscillograms from a reference device (EVOM) and the self-assembled voltammeter for comparison.

NOTE: In this case, the data was recorded with a Digital Storage Scope HM 208. Being a very basic digital oscilloscope, the image could be internally digitized (frozen) but had to be plotted using an analogue PM 8143 X-Y recorder. The image was subsequently scanned.

4. Cell cultivation and TEER measurement

4.1. Seed Human Choroid Plexus Papilloma (HIBCPP) cells on cell culture filter inserts with a pore size of 3 µm in DMEM/F12 (see **Table of Materials**) containing 10% fetal calf serum[9]. Grow the cells at 37 °C in a water saturated atmosphere containing 5% CO_2 as described by Dinner et al.[9].

4.2. When the filters reach an impedance of 70 Ω · cm^2, change to serum-free DMEM/F12 and define the timepoint as Day 0.

4.3. Connect the electrode to the RJ14 port of the self-assembled voltammeter and plug in the USB power supply. Set the multimeters to AC voltage mode (mV) and AC current mode (µA), respectively.

4.3.1. Alternatively, connect the electrode to a commercially available reference device and turn on according to the manufacturer’s instructions.

4.4. Sterilize the electrode in 80% ethanol for 10 min and equilibrate in the appropriate medium for another 10 min.

4.5. Put the electrode in both compartments of a cell culture filter insert system (the longer part of the electrode in the lower compartment and the shorter part in the upper compartment) containing a HIBCPP cell layer until the measurement values remain constant.

4.6. For a reference device, note the impedance directly or calculate the impedance according to Ohm's law (R = U/I) for the self-assembled voltammeter. Be aware that electrode angle affects the measurements.

4.7. Repeat the TEER measurement (steps 3−6) from Day 0 until Day 4.

RESULTS:

To compare the operation of a self-assembled voltammeter with its commercially available counterpart, a voltage oscillogram of both devices was recorded.

As shown in **Figure 2A**, the reference instrument generated a square wave signal with an amplitude of 80 mV and an oscillation time of 80 ms, which corresponds to a frequency of 12.5 Hz, when operating on-load with a 1 kΩ test resistor.

In contrast, the microcontroller of the self-assembled device switched the supply voltage to a square wave signal with an amplitude of 5 V (**Figure 2B**) if no pre-resistor was set in. It became apparent that the resulting current destroys any barrier function and is not applicable for cell culture experiments (data not shown). A further issue is that, in this setup a 1 kΩ test resistor caused an overload with a resulting decline of voltage (**Figure 2B**). Additionally, the effective oscillation time of the microcontroller was 60 ms (frequency = 16.7 Hz) and thereby differed from the programmed delay time due to the inaccuracy of the time emitter. If a 120 kΩ preresistor was installed, the amplitude decreased to a value of 40 mV, which was suitable for cell culture (**Figure 2C**). As seen in the oscillogram, signal-to-noise ratio was considerably impaired (**Figure 2C**) but did not affect measurements noticeably.

Both devices were used to determine the impedance of an artificial blood-cerebrospinal fluid barrier (simplified circuit diagram shown in **Figure 2D**). HIBCPP cells were cultivated on cell culture filter inserts and TEER was measured over 6 days: starting one day before cells were moved to serum-free conditions (Day -1) and up to 4 days after changing the medium (Day 4). All measurements were done in quadruplicates using four HIBCPP filters prepared in the same manner. Similar values were obtained for the reference instrument and the self-assembled voltammeter (**Figure 3**). Measurements were reproducible, and standard deviations were within the same range. TEER values ranged from 20−550 Ω · cm^2. Using 0.33 cm^2 filters, this equates to an absolute impedance of 83−1,660 Ω.

FIGURE LEGENDS:

Figure 1: Layout diagram of a basic volt-amperemeter for TEER measurement.

Figure 2: Oscillograms and measurement setup. (**A**) Commercially available EVOM. (**B**) Self-assembled voltammeter without pre-resistor. (**C**) Self-assembled voltammeter with 120 kΩ pre-resistor. d) Circuit diagram of measurement setup. Note that $C_{electrode}$ only appears in the electrical circuits when bipolar systems are used.

Figure 3: TEER measurements of HIBCPP cell layers on cell culture filter inserts before switching to serum-free culture medium (Day -1), on the day of switching (Day 0), and up to 4

days after (Days 1–4). Error bars indicate the standard deviation of the four HIBCPP filters that were prepared in the same manner.

DISCUSSION:

Before a self-made voltammeter can be used in a daily routine, it is essential to check the device for proper function. In our case, a half-time of oscillation of 40 ms (12.5 Hz) was programmed, but the effective oscillation time turned out to be 60 ms (16.7 Hz). This inaccuracy of the microcontroller's time emitter had no detectable impact on TEER measurements. It might be best to determine the actual frequency using the frequency setting of one of the multimeters. If any deviation is found, the source code can be adjusted accordingly. Further, it is strongly recommended to check whether a test resistor or other defined setups give correct and reproducible results. If working with artificial cellular barrier systems, it might be best to always correlate molecule flux with impedance measurement.

In this case, the applied current was limited using a 120 kΩ pre-resistor. Assuming that typical TEER values range from 100 Ω–2,000 Ω, the voltage drop across the cell layer can be calculated to be 4–83 mV. A TEER of 1 kΩ was simulated by a test resistor and the resulting potential drop was confirmed to be 40 mV (**Figure 2C**).

Commercially available devices often provide a measurement range switch to toggle the pre-resistor and thus limit the output current to different values. In this case, it is feasible to install different pre-resistors or to even replace the resistor with a potentiometer.

The shown setup represents a cost-effective alternative to commercially available instruments for TEER measurement. Values that have been measured with the self-assembled voltammeter were comparable to the reference device over a broad range. The same is true for the standard deviations. The noise in the square wave signal did not affect measurements notably. The protocol can support scientists who are restricted by limited financial resources or who want to perform preliminary experiments at low costs.

Further, the microcontroller can be easily programmed to different output frequencies. This may be beneficial, as the apparent impedance consists of R_{medium}, R_{TEER}, as well as the capacity $C_{cell\ layer}$[26] (**Figure 2D**). Additionally, $C_{electrode}$ appears if bipolar systems are used, whereas the influence from the electrode polarization impedance is reduced in tetrapolar systems. This means that the measured impedance will be dominated by R_{TEER} at low frequencies and, in bipolar systems, by the capacity of the electrodes, whereas at high frequencies the total impedance converges to the resistance of the medium[26,27]. In between, the impedance is influenced by $C_{cell\ layer}$, which is therefore accessible using electrical impedance spectroscopy[28].

ACKNOWLEDGEMENTS:

The authors would like to thank Herman Liggesmeyer and Marvin Bende for their expert advice in electrotechnics and informatics.

DISCLOSURES:

The authors have no competing financial interests or other conflicts of interest.

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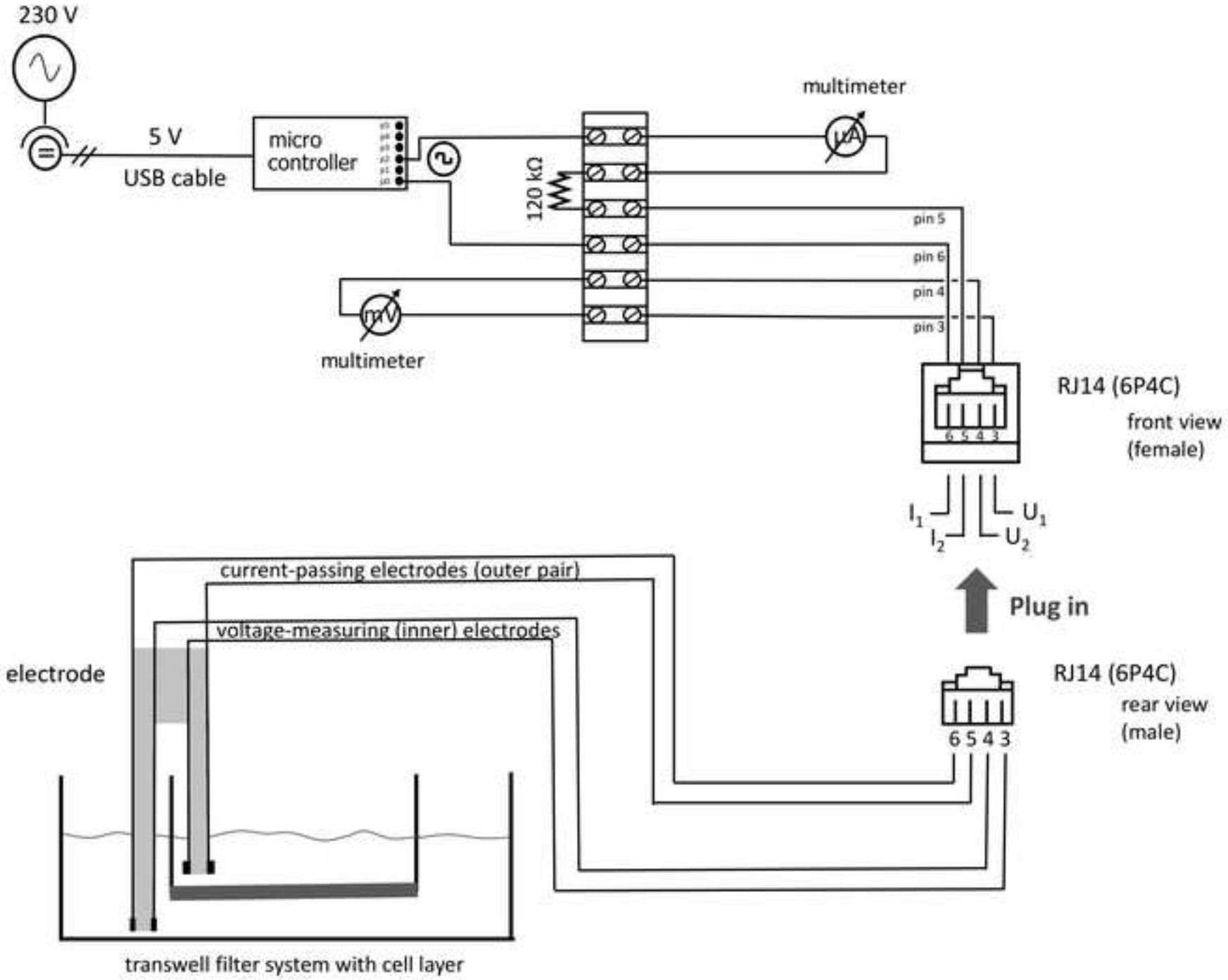
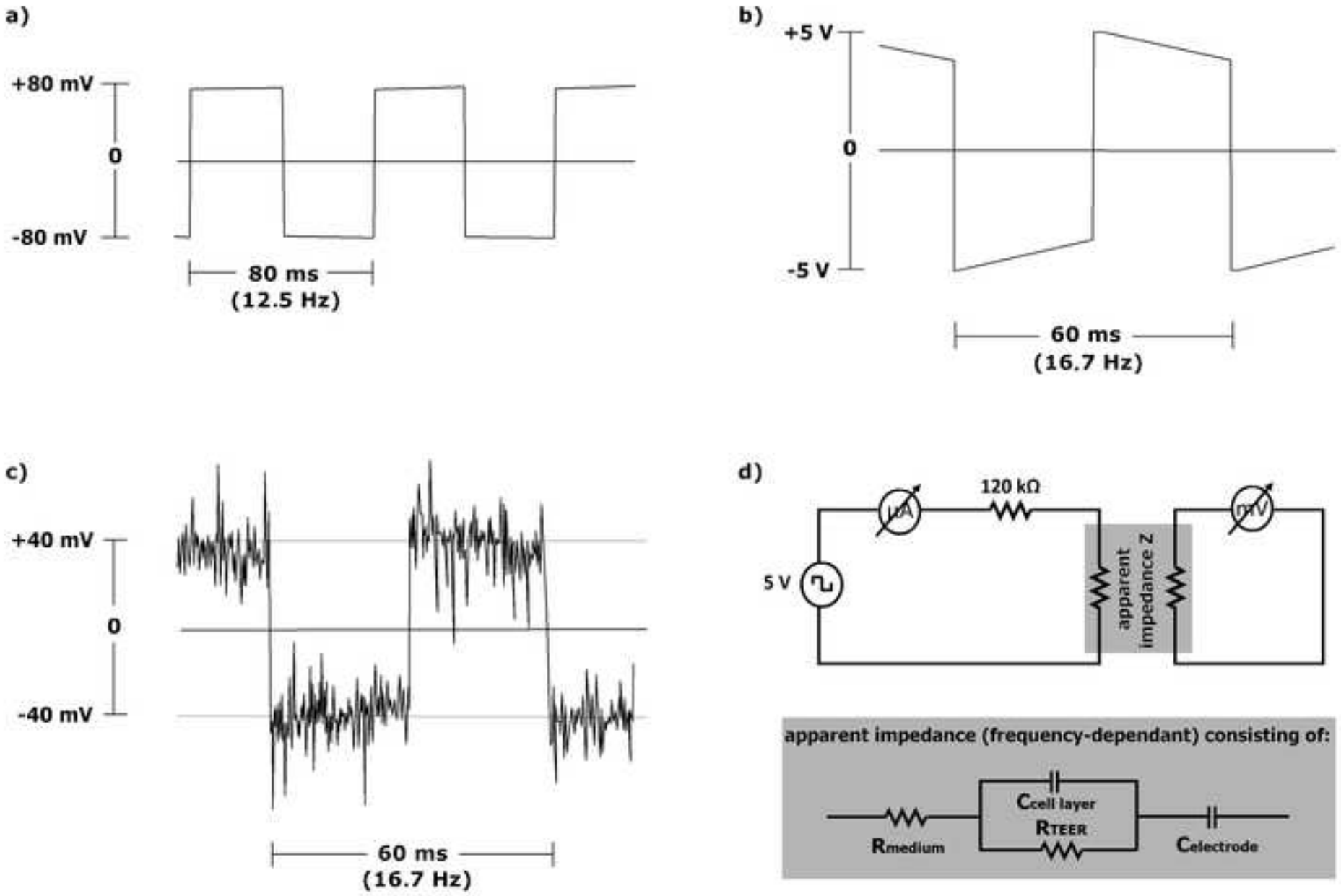
Figure

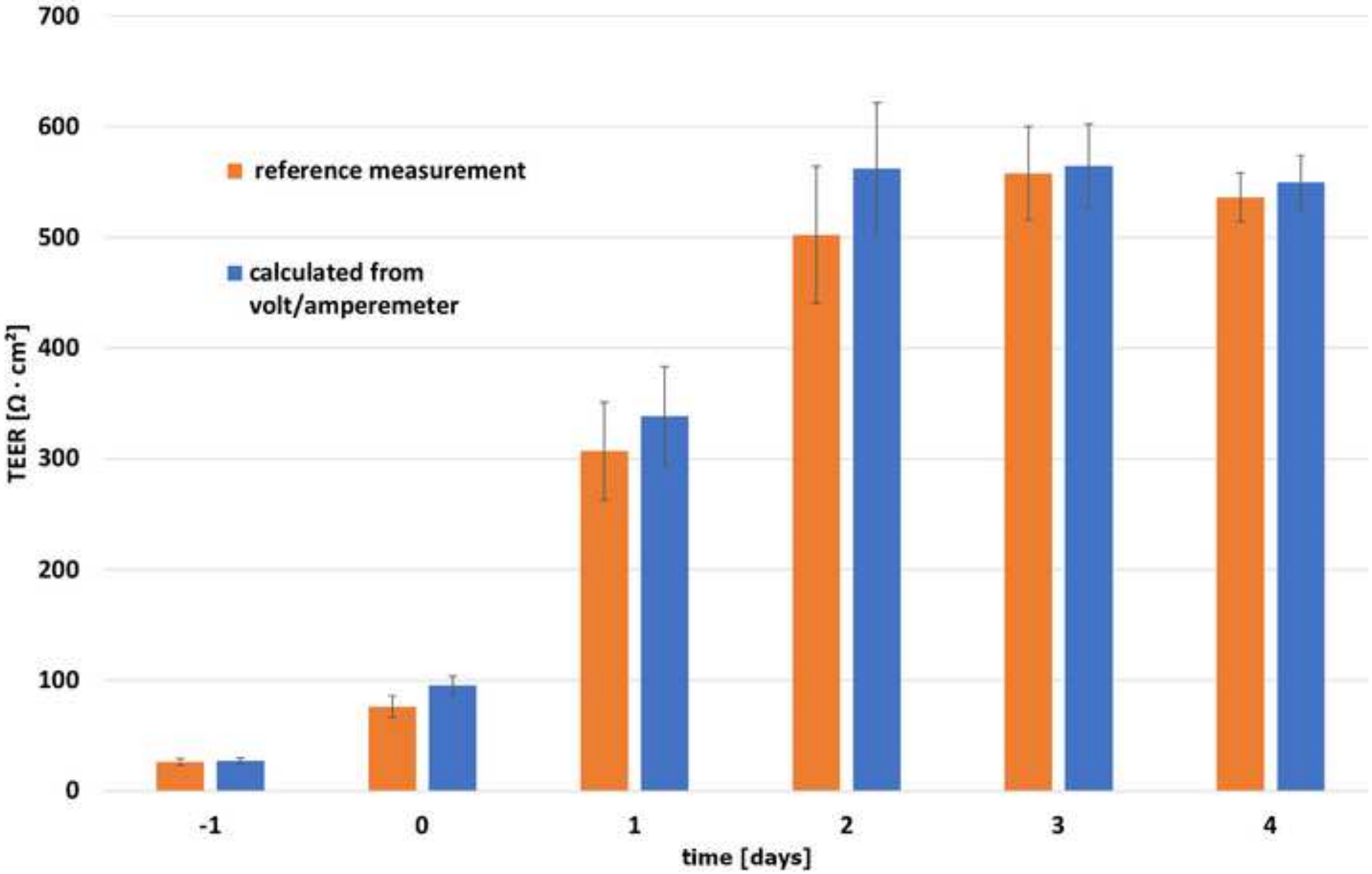


Figure



Figure

Click here to access/download;Figure;Figure 3 - Impedance measurement.psd



Name of Material/Equipment	Company
120 kOhm resistor	
Banana plug cables	
Cables	
Chopstick electrode	Merck Millicell
Chopstick electrode (alternative)	WPI World Precision Instruments
Crimping tool	
Digispark / ATtiny85	AZ-Delivery Vertriebs GmbH
DMEM:F12	Gibco (Thermo Fisher)
Fetal calf serum (FCS)/Fetal Bovine Serum (FBS)	Life Technologies
Filter inserts 3µm translucent	Greiner Bioone
HIBCPP	
Insulation stripper	
Luster terminal	
Oscilloscope	HAMEG
Plotter	PHILIPS
Software Arduino	https://www.arduino.cc
Soldering iron	
Soldering lugs	
Telephone cable with RJ14 (6P4C) connector	
Test resistor	Merck Millicell
True-RMS multimeters	VOLTCRAFT
USB charger	
USB extension cord	
Voltohmmeter for TEER measurement	WPI World Precision Instruments
Voltohmmeter for TEER measurement (alternative)	Merck Millicell
Wire end ferrules	

Catalog Number	Comments/Description
	General (generic) equipment
	General (generic) equipment
	General (generic) equipment
MERSSTX01	
STX2	
	General tool
Digispark Rev.3 Kickstarter	
31330038	
10270106	
662631	
	Hiroshi Ishikawa / Horst Schroten
	General tool
	General (generic) equipment
Digital Storage Scope HM 208	
PM 8143 X-Y recorder	
Arduino 1.8.9	
	General tool
	General (generic) equipment
	General (generic) equipment
MERSSTX04	
VC185	
	General (generic) equipment
	General (generic) equipment
EVOM	
ERS	
	General (generic) equipment

Dear Dr. Steindel,
dear Sir or Madam,

thank you for your editorial comments concerning the submission JoVE60087R1 with the title "A simple approach to perform TEER measurement using a self-made volt-amperemeter with programmable output frequency".

We have addressed all issues as follows:

- **0:00-0:39 - While the speaker is visible on screen, the audio appears not to be synchronized at various points. It appears that the audio was recorded separately and dubbed over the video. If it exists, the live audio should be included instead of the dubbed audio for the introductory statement.**
  You have correctly recognized that the audio had been dubbed over the video. By doing so, we wanted to provide a slightly better sound quality than in the live audio. Anyhow, we understand that this was at the expense of synchronicity. In the latest version for the introductory statement the live audio is included instead of the dubbed audio.

- **2:17, 3:23 - 'ATtiny85' and 'Digispark' are mentioned here in the narration. These are brand names that should be replaced with more generic terms.**
  At 2:17 the narration was changed to 'An 8-bit microcontroller on a USB development board will then be used to generate a square wave current'. At 3:23 and 3:47 'Digispark' has been removed from the narration.

- **Figure 3 in the video has 'Reference measurement' and 'calculated from…'; please be consistent with respect to capitalization. Also, please explain the error bars in the legend.**
  Capitalization has been corrected ('reference measurement') in the video (figure 3 at 7:13). This issue was already fine in the manuscript.

  Additionally, we found another inconsistency in capitalization: 'multimeter' is now written in lower case (figure 1 in the manuscript and in the video at 3:13 & 5:03).

  We added the following sentence to the legend of figure 3: "All measurements have been done in quadruplicates. Error bars indicate deviation of four HIBCPP filters which had been prepared in the same manner."

- **Assembly… 3: The video also has the option of soldering the cable directly to the pins.**
  We changed Assembly…3 to "Strip the end insulations of two short cables. Solder one side per cable either directly to pins 0 and 2 of the microcontroller, or to soldering lugs, which in turn are clipped on the respective pins. Crimp the other ends to wire end ferrules and connect them to a luster terminal as depicted in figure 1."

- **Assembly…. 3 and 6: The units of current (as well as using AC) and voltage are mentioned in the video but not here.**
  We already mention the settings of the multimeter (units and AC) in section Cell cultivation and TEER measurement…3a

  Additionally, we added this information
  - to Assembly…6: The first multimeter will be used to measure current in µA (note that AC mode has to be set explicitly).
  - and Assembly…7: Finally, link the second multimeter (which will be used to measure the transepithelial voltage drop in mV) via the luster terminal to pins 3 and 4 of the RJ14 connector.

- **Assembly…: The video shows how to mount this.**
  We added Assembly…8: "If desired, mount the installation in a chassis of your choice."

With the latest revision, we hope to meet all requirements for publication and look forward to your answer.

Yours sincerely,
Stefan Mogk

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Supplemental Coding Files

Supplemental Coding File 1 - 12.5 Hz.txt

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Supplemental Coding Files

Supplemental Coding File 3 - max. frequency (70 kHz).txt

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