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A Low-Cost and Customizable TEER Meter for the Measurements of Cellular Barrier Integrity

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A low-cost and translational TEER (trans-endothelial/epithelial electric resistance) meter was designed, fabricated, validated, and applied in this paper. TEER is a critical tool to quantitate the integrity of biological barriers. Commercially available TEER meters are expensive (thousands of dollars) with low customization capability. Using Arduino, an open-source hardware and program that are used to control electronics, we fabricated the TEER meter that costs ~$50 to purchase the parts and 2 hours to be constructed. Robust characterization and validation shows that the meter can accurately measure TEER values between 132 and 82,500 $\Omega \cdot cm^2$ with <3% errors, which covers the reported TEER ranges based on a literature study we conducted. The temporal resolution, the measurement duration, and the electrode configurations of meter are also customizable. We successfully applied the meter to measure TEERs of endothelial cell monolayers, finding that cells treated with histamine have lower TEER values compared to untreated cells (793.4 ± 190.5 $\Omega \cdot cm^2$ vs. 3027.5 ± 664.4 $\Omega \bullet cm^2$; $p < 0.001$), which is consistent with literature results. We further validated the TEER measurement by showing that histamine increased the intercellular gap from 2.34 ± 0.12 µm to 5.49 ± 0.17 µm, causing leakier endothelial barriers and thus lower TEERs. In conclusion, we report for the first time a low-cost Arduino-based TEER meter capable of accurately measuring TEERs in the relevant range. We also include detailed tutorials in the supplementary information to promote the translation of the technology.

A Low-Cost and Customizable TEER Meter for the Measurements of Cellular Barrier Integrity

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INTRODUCTION

Endothelial and epithelial cells are the main component of biological barriers with endothelial cells on the inner linings of blood vessels and epithelial cells forming protective wrappings along the exterior of vital organs such as the lungs and liver[1-3]. These cells form a monolayer with tight intercellular junctions via proteins including the occludins, claudins and tetraspanins[4-6]. Tight junctions are critical to maintain the whole-body homeostasis by controlling the permeability of substances across the barriers that separate the luminal and abluminal sides of the body[4]. Therefore, the permeability of endothelial/epithelial cell monolayers have been widely studied in fundamental physiological and pharmaceutical (e.g. drug delivery across the barriers) research[7, 8]. Transendothelial/epithelial electrical resistance (TEER) is a prevailing technique to quantitate the permeability of the cellular barriers due to its non-destructive characteristic and the relatively simple setup[9, 10]. TEER measures the electrical resistance across a cell monolayer cultured on a porous structure (e.g., a membrane)[11]. Without cells, the porous membrane allows for charge exchange across it and thus the resistance is relatively low. With a tightly packed monolayer of cells, a high resistance value will be obtained because the barrier prevents the charge exchange while loosely packed cells will reduce the charge blockage causing resistance drops[12]. Although TEER devices directly measure the resistance across a cell barrier, TEERs are typically reported as the resistance flux across the area with a unit of $\Omega \cdot cm^2$, which is calculated by multiplying the measured resistance by the area of a cell monolayer[9-13].

Although commercially available, TEER devices are expensive (thousands of US dollars) and the electrode configurations are not customizable to fit a tissue/cell culture of specific dimensions and/or geometry. Consequently, a TEER meter that is low-cost, customizable and accessible to every laboratory is needed, which is the motivation of the presented work. Because of the simplicity of the principles behind electrical resistance measurements, it is feasible to fabricate a TEER device with limited resources. Here, we report a simple, low-cost, customizable, translational, and accurate TEER sensor controlled by Arduino. Arduino is an open source platform with an easy-to-use software that can be applied to control electrical devices via an integrated board[14]. Arduino boards contain an Atmel AVR processor[14] and can be easily purchased as preassembled units (<$15). While there are other microcontroller boards, what makes Arduino unique is the open source nature of both its hardware and software. Arduino's free open-source software is based off the C/C++ languages but in an easy-access format[15]. It communicates to Arduino boards to enable the acquisition of both digital and analog inputs/outputs executing functions that can manipulate various electrical devices. Some recent applications that employ Arduino include a humidity monitoring sensor network for an entire floor of a building, a web-accessible soil irrigation system with a temperature and soil moisture monitor, and an automated system for a warehouse that monitors security[16-18]. Arduino also offers a database full of tutorials and user-uploaded projects to facilitate streamlined designs and fabrications[14].

Specifically, in this paper, we demonstrate for the first time the fabrication of a TEER meter using Arduino, which costs ~$50 to purchase the parts and less than 2 hours for construction. We also include a detailed tutorial on the hardware and the programming in the SI to promote translation of the technology. A protocol to customize TEER electrodes is also included. Standard resistors were applied to characterize the accuracy of the meter, which can measure any resistance between 400 and 250,000 Ω with an error less than 3%. With the Arduino programming, automated measurements with desired temporal resolution can be set. Consequently, we used the meter to detect the TEERs across endothelial monolayers formed in transwell membrane inserts continuously for 1 hr at 1-min intervals, finding that cells treated with histamine have significantly lower TEER values that those without the treatment, which is consistent with literature[12] and is confirmed by our further analyses on the intercellular gap sizes. Overall, we present a novel TEER meter that is low-cost and customizable (electrode configuration and measurement settings). With our tutorial in the SI, we believe this technology will be translational to broadly enhance biological barrier studies.

EXPERIMENTAL

Construction of the TEER device

The electrode. TEER measurements need a "chopstick" electrode with two leads that can be placed on each side of a cellular barrier. We developed two methods to fabricate such an electrode with limited resources/budget. The first method uses a spacer fabricated by 3D-printing, which is a 15mm (diameter) disc. There are two holes of 0.8 mm diameter that are 5 mm apart around the center of the discs, where the two leads of the electrode will be placed. Stainless-steel wire (0.032 in., Mcaster-Carr, NJ, US) was cut into 6 cm sections and inserted through the holes in the spacer. The heights of the two steel leads across the disc were 13 mm and 16mm, respectively. Using epoxy, both wires were immobilized on the disc. To avoid metal cation contamination during a TEER measurement, the steel leads of the electrode were dipped in carbon conductive ink (MG Chemicals, Ontario, CA) followed by air drying for 24 hrs to coat a layer of carbon on the steel surfaces.

The second design can be used in laboratories without access to 3D-printers. Three polystyrene sheets (250 µm thick; Shrinky Dinks, MI, US) were stacked and the edges were fused together via heating and melting. Two holes were punched using a heated metal needle through the sheets to house the stainless-steel electrode leads. The same dimensions, epoxy and carbon coating protocols as aforementioned are used to finish fabricating the electrode.

The circuit. All parts needed to construct the meter were purchased on Amazon.com unless otherwise noted. An Arduino UNO board was used, which enables 5V power supply and analogue reading of the voltage across a resistor. A jump wire coming out of the 5V port on the board connects to the measuring electrode (with two leads) and a standard resistor of 117 kΩ in serial, with another wire going to the ground port (GND) to form a closed circuit. Across the standard resistor from the ground, a third wire is connected and ends in the A0 analog port. Between A0 and GND, the voltage drop across the standard resistor can be measured.

Programming of the Arduino

The program was written in the Arduino IDL (the free Arduino software to program and display data). The coding is shown with detailed explanation in the results and discussion section (**Fig. 3**). We also included a tutorial instruction in the SI to further explain the coding, and demonstrate how the program can be changed to customize detection duration and temporal resolution etc. The coding is broken down into 3 main parts: defining of variables, the setup of the Arduino microcontroller, and the method by which the inputs from the circuit are converted into resistance measurements.

Quantitative characterization of the TEER meter

This is to ensure the meter can read resistance values accurately. Resistors of known resistances were connected to the detection electrode of the meter one by one. A variation of the programming was applied to take measurements every 5 sec for a total of 1 min (12 measurements). The signals from one resistor were averaged and then compared to the known value (measured by a calibrated multimeter). The measured values were plotted as a function of the known values. We also calculated the recovery of the measurement values, which equals to (the measured value/the known value) X 100%.

Applying the meter to measure TEERs across endothelial cell monolayers

Cell culture in transwell membrane inserts. Bovine pulmonary aortic endothelial cells (BPAECS; ATCC, VA, US) between passages 4 and 6 were used throughout the study. Buffered DMEM media–Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, MA, US) containing 10% fetal bovine serum, (MilliporeSigma, MO, US) and 1% Penicillin Streptomycin (Thermo Fisher Scientific, MA, US) was used for all cell culture experiments. The 24-well plate membrane inserts (0.4 µm pore size; Corning, NY, US) were precoated with 0.3 mg/mL of collagen for 24 hours, followed by adding 300 µL of BPAECs suspension (in DMEM media, 150,000 cells/mL) into each insert. The cells were cultured in a 37 °C incubator (5% CO2, humid) for 4 days when a confluent monolayer is formed.

TEER measurements of untreated endothelial cells. 300µL of DMEM media was placed in an insert and 1mL of the same media in the well outside of the membrane, the short lead of the electrode was placed in the insert while the longer one was outside. This setup allows the flow of current through the endothelial monolayer so that the resistance can be measured. Placed in a 37°C incubator, the Arduino was started to take the measurements at 1-min intervals for 1 hour.

TEER measurement of endothelial cells with histamine treatment. A histamine (MilliporeSigma, MO, US) solution of 20 µM was made in DMEM media. Before the study, the previous media in an insert and the corresponding well were replaced by the histamine solution, followed immediately by placing the electrode in and starting the measurement.

Verification of the endothelial TEER measurements with microscopy. Immediately after each trial, the cells were fixed with methanol (VWR, CT, US) for 10 minutes at -20°C. Then the fixed cells were washed with HEPES buffer (MilliporeSigma, MO, US) and stained with a 0.5% cystal violet (VWR, CT, US; w/v) solution for 10 min at room temperature[19, 20]. The cellular monolayers were then imaged on a standard light microscope and using ImageJ, the intercellular gap was measured (n=70 for each trial).

Data, calculations, and statistics. All TEER measurements were repeated three times. The Arduino IDL enables real-time visualization of the recorded data in a window called serial display. The data were recorded in two columns controlled by the program: time (min) and resistance (Ω). The data columns can be simply copied out of the serial monitor (in .txt format) and pasted in Microsoft Excel for graphing. TEER values are commonly reported as the flux of resistance across a known area in the unit of $\Omega \cdot cm^2$ and thus, the resistance values recorded by our meter were multiplied by 0.33 cm^2, which is the area of a 24-well membrane insert, as the final readings. The TEER values were plotted as a function of time. The student-t test was applied to compare data groups, and significant difference is only valid when the p value is smaller than 0.05.

RESULTS AND DISCUSSION

TEER measurement is a critical tool to study biological barrier integrities. Commercial TEER meters are expensive with limited customization capability. We developed a low-cost yet accurate TEER meter which also enables customizable electrodes and measurement settings (duration, temporal resolution etc.). The construction, characterization/validation, and applying the meter to monitor endothelial barrier TEER are discussed below.

Construction of the TEER meter

Electrode fabrication

A common configuration of TEER electrode utilizes two prongs/leads like the shape of a pair of forceps, which can be placed on both sides of a barrier to measure the resistance. Although commercial electrodes are available, they are preassembled with limited customizability for specimens of specific shapes and dimensions. Here, we present two fabrication techniques to obtain customizable TEER electrodes. As shown in **Fig. 1A**, a disc of

15mm diameter was 3D printed with two holes (0.8 mm diameter; 5 mm apart) that fits 0.032 in. (0.8 mm) stainless steel wires. The two wires will serve as the leads of the electrode and the shorter one is protruding 13 mm across the discs while the longer one is 16 mm. The 5 mm space between the two leads of the electrode was determined optimal so that the electrode can fit a 24 well membrane insert (with the shorter lead in the insert while the long one outside) shown in **Fig. 1C**. To avoid iron cation contamination during the electric detection, the two leads of the stainless-steel electrode were coated with a layer of conductive carbon

We also provide a fabrication protocol without using a 3D-printer. As **Fig. 1B** demonstrates, three squares were cut out of a Shrinky Dinks sheet and soldered together to create a rigid support in lieu of the 3D printed disc. The two holes that the leads thread through were punched by a heated steel wire. The stainless-steel leads that make up the electrode were affixed onto the support using epoxy. This electrode construction protocol not only emphasizes the simplicity, but also displays the customizability of the electrodes. They can be manufactured to any desired dimensions and be fastened to an experimental setup with ease. We used stainless steel as the electrode material due to ready availability, but other materials such as gold and platinum can be used as well–the connected leads simply need to be conductive. Also, other dimensions (e.g., microelectrodes) and/or shapes (e.g., planar electrodes) can be fabricated to make the electrode.

<u>The TEER meter fabrication</u>

The principle of this resistance measurement is based on the voltage splitting principle derived from the Ohm's Law[21]. As shown in **Fig. 2A**, there are two resistors (R1 and R2) in a serial circuit across a 5V voltage. The voltage drop across the resistors are defined as V1 and V2. Based on the Ohm's law, V1 and V2 splits the input voltage (5V) contingent on the magnitude of the resistances, showing in Equation 1:

$$\frac{V1}{V2} = \frac{R1}{R2} \qquad or \qquad R2 = V2\frac{R1}{V1} \qquad \textit{Equation 1}$$

In a real application, R2 will be the resistance across a sample (cell monolayer) and R1 will be a known resistor in the circuit. Therefore, as long V1 and V2 can be measured, the value R2 can be elucidated based on Equation 1. We used a 117kΩ resistor as R1; the resistance was chosen because of the low current–previous research has proven that with such a resistor in a 5V circuit, the corresponding current does not affect endothelial cells[22].

Figs. 2B and **2C** show the schematic and real wiring of the TEER meter. A jump wire coming out of the 5V port on the Arduino board connects the TEER electrode (with two leads; R2) and the known resistor (117 kΩ, R1) in serial, and the circuit ends at a ground port (GND) on the board. Across R1 from the other side of the ground wire, another wire is connected to the A0 analogue port on the Arduino board, which will measure the voltage drop between this wire and the ground wire (V1). Otherwise speaking, A0-GND is an analogue voltage meter. The voltage drop across a sample (V2) can then be calculated as $V_2 = 5V - V_1$.

Programming of the TEER meter

Fig. 3 is the program to measure R2 (TEER) based on Equation 1. There are three main modules of the codes: defining parameters, Arduino setup, and the measurement.

Defining parameters. Before anything else can be done all the variables that will be used need to be defined. While variables can be defined in a function, we chose to define the variables globally to carry their definitions over the rest of the program. The following variables are defined as an integer variables: the analogPin()=0 which defines that the analog pin A0 will be used (there are 6 analogue ports numbered 0-5); the pinReading variable that will hold the voltage value read from the analogue port 0; the applied voltage to the circuit is define as V0, which is 5V; the variable t is the timestamp of each reading with the initial value being t=0; and the i variable serves as a loop controller for how many measurements are will be taken.

The following variables are defined as floating numbers: the voltage V1 which is the voltage drop across the known resistor (R1; 117,000 Ω) read from the analog pin on the board; the voltage V2 which is the voltage across R2 (the resistance of the cell monolayer in solution).

The numerical variable are defined as integers (int) or float numbers (float) based on their specific roles in the measurement. Integers are for counting and comparisons purposes, for example, the integer variable i that counts the number of measurements. A float number uses decimal places and is commonly used for calculations. In our case, the variables that are either constant or will be used for printing in the serial monitor are recorded as an integer and the float variables are used in the mathematical calculations for accurate results.

Arduino Setup. The Serial.begin() function sets the data rate in bits per second that the Arduino transmits to the serial monitor, which is the window that displays the results the program detects. The default Arduino data exchange rateis 9600 bit/sec, so the serial monitor is set up to 9600. The Serial.printline() function will print/display two columns in the serial display defined by the content in the parenthesis: time as the first column in the unit of min and resistance as the second one in the unit of Ohms (Ω), and a ";" will be printed as the separator between the columns.

The measurements. The resistance measurements are calculated and printed to the serial monitor in a void loop. A void loop in Arduino circulates until the condition is voided. First in the loop, the PinReading() functions reads from the analog pin A0 on the Arduino board, which is the analogue voltage value across the known resistor (R1). Next, the if(i<=61) defines that 61 measurements will be taken (1 min per measurement for 1 hr including the 0 point).

R1 and V0 in the circuit are known, which are 117,000 Ω and 5V, respectively, and as discussed in Equation 1, to measure R2 (sample), V1 and V2 needs to be known. The PinReading()=(analogPin) reads V1 in the format of how many parts out of 1024. A total of 1024 parts equals 5V and thus PinReading()/1024 X V0 (which is 5V) results in V1. Because R1 and R2 are in serial, V2=V0-V1 and R2 can then be calculated. The next section of this part is to print the data. The measurement timestamp t will first be printed, followed by ";" and then the measured R2. A delay of 60,000 millisecond (the coding "delay(60000)") or 1 min is executed before the next measurement (this temporal resolution is customizable). Subsequently, another timestamp that is 1min later is assigned to the t variable (the coding "t=t+1") and the next measurement loops through. We included a more detailed explanation of the coding in the SI and a tutorial about how to change temporal resolution and detection duration (**Fig. S4 and Table S1**).

Validation of the TEER meter

A TEER meter is essentially a resistance meter. Therefore, standard resistors were measured by the TEER meter and the measured values and the true values were compared. **Fig.**

4A shows the plotted measured values as a function of the true values, with a linear regression curve of y = 1.0073x - 34.528. The small intercept and the slop that is close to 1 indicates quantitative recovery of the standard resistors. Indeed, further analyses reveals that when above 400 Ω, the variance between the measure and the true resistances are within ±3%. In other words, the TEER meter can measure resistance in the range of 400 and 250,000 Ω accurately with errors <3%. Because TEER is commonly measured across a 24 well plate membrane inserts (0.33 cm^2) or smaller microfluidic interfaces[23-25], the equivalent quantitative TEER range our meter can be converted to 132-82,500 $\Omega \cdot cm^2$, which covers the reported values based on a literature study we performed (**Fig. 4C**)[9, 23-29].

Measuring endothelial TEER using the meter

Endothelial cells are responsible for the exchange of molecules such as drugs and nutrients between blood and other tissues[9]. TEER measurement is an indispensable assessment of endothelial barrier integrity in physiological and pharmaceutical studies[1, 6, 10, 11]. Therefore, we chose endotheial cells as a model to test our TEER meter. The cells were cultured in 24-well plate transwell membrane (0.4 µm pores through) inserts. As a control, histamine was applied to treat the endothelial cells, which is known to be able to break the endothelial integrity and thus make the barrier leakier[13, 30, 31].

In addition to the simple construction, the TEER meter is easy to be set up by a cell culture incubator. As shown in **Fig. 5A**, the Arduino board was taped to the side of an incubator while the wires connected to the electrode can be placed inside. Because the jump wires are thin (1 mm) and there is a flexible gasket inside the incubator door, we did not see temperature or CO_2 drop during the experiments. Once the electrode was placed in the membrane insert and the well, the serial reading will be started in the Arduino IDL installed in a laptop computer next to the incubator.

Shown in **Fig. 5C**, The TEER of the endothelial monolayer that was not treated with histamine was consistent over the course of 1 hour with the magnitude around 3027.5 ± 664.4 $\Omega \cdot cm^2$ (average of triplicate experiments). However, when treated with histamine, the TEER of the endothelial cells immediately dropped to 1744.5 ± 505.6 $\Omega \cdot cm^2$ (mean of 3 replicates ± S.E.M.)

within 10 min and keeps decreasing during the 1-hour study to a final value of 793.4 ± 190.5 $\Omega \cdot cm^2$. Statistics show a p value < 0.01 for all the data points attained (every minute for 1 hr), suggesting that histamine significantly reduces the TEER or endothelial barrier integrity, which is consistent with literature[32, 33].

In order to verify that the TEER drop was caused by histamine, the cells were immediately fixed and stained with crystal violet after the 1-hour experiments. Images of the cell monolayer were then taken on a bright field microscope. It appears that the intercellular gaps between endothelial cells treated with the histamine are bigger than those of the untreated cells (**Fig. 5B**). We then quantitated the gaps using ImageJ finding that the average intercellular gap between the cells untreated was determined to be 2.34 ± 0.12 µm, while the cells treatment with the histamine have significantly enlarged gaps (5.49 ± 0.17 µm; p<0.001), as shown in **Fig. 5D**. This data proves that the histamine causes larger intercellular gaps, which must lead to lower TEER values. The results also successfully validate our TEER meter.

Conclusion

In this paper we report the design, fabrication, and application of a low cost yet accurate TEER meter using Arduino and a few other easily accessible materials. In total, it costs ~$50 (**Table 1**) to purchase the parts and about 2 hours to construct the meter including the electrode. In the span of 1 day, a fully functioning and characterized TEER meter can be made ready to measure real samples. Also, our design allows for customization in terms of electrode material, dimensions, geometry, and measurement settings (e.g., temporal resolution) etc. With these advantages, the fact that Arduino is open-source, and our detailed tutorial in the SI, we believe that this TEER meter is readily translational to broadly enhance biological barrier research.

FIGURES AND CAPTIONS

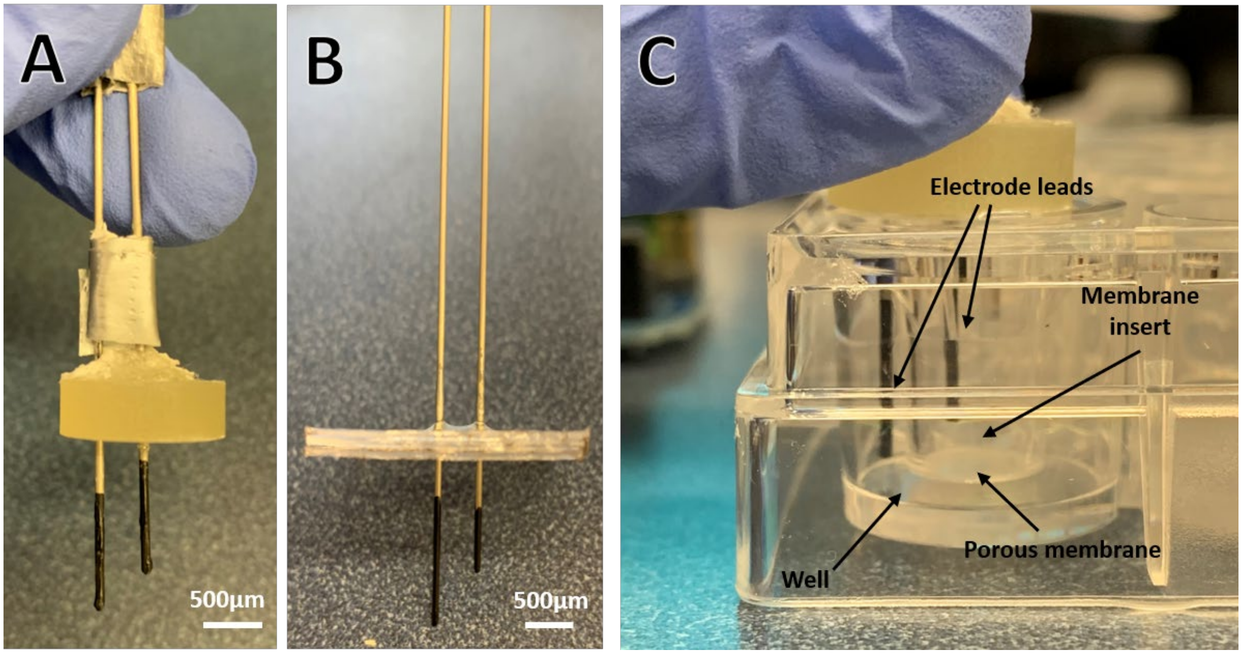

Figure 1. Fabrication of the electrode. Chopstick electrodes with two leads that can be placed on both sides of a cell monolayer are used in TEER measurement. **(A)** shows a 3D printed spacer to house two steel rods coated with carbon as the electrode. **(B)** We also offer a design without using a 3D printer: plastic pieces with punched holes can be used to house the electrodes. Epoxy may be needed to immobilize the electrodes. **(C)** Electrode placement across a transwell membrane insert. A layer of endothelial cells will be cultured on the top side of the membrane. The shorter lead is placed within the insert while the longer one is outside, so that the resistance across the membrane (where cells will grow) can be detected.

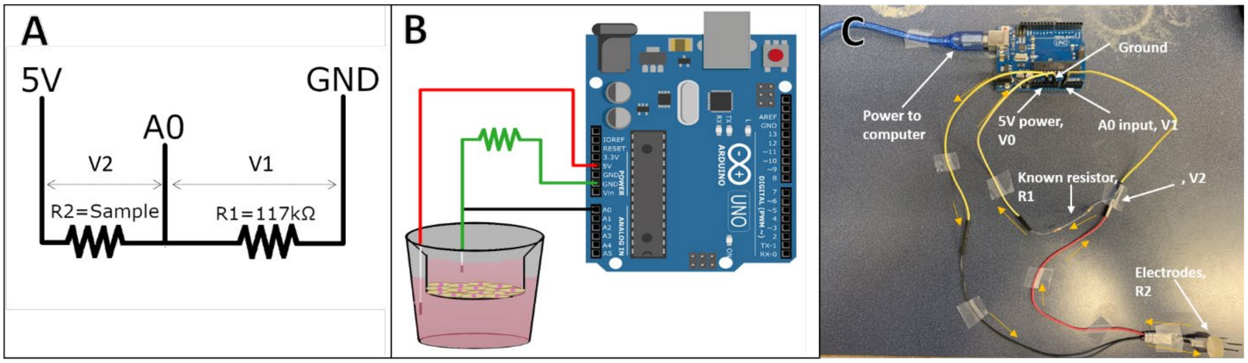

Figure 2. The principle and design of the TEER meter. **(A)** The voltage splitting principle: with two resistors in a serial circuit, the voltage drop across each resistor is proportional to the resistance, or R1/R2=V1/V2. If the total voltage applied to the circuit and R1 are known, and V1 can be detected, R2 can be calculated. **(B)** The schematic of the meter fabrication. A sample (resistance across a monolayer of cells in the well) and a 117 kΩ resistor are connected in serial starting from the 5V power port and ending on a ground (GND) on the Arduino board. The voltage across the 117 kΩ resistor will be measured between the A0 analogue input (black line) and the GND, so that the voltage across the sample can be calculated, and thus the resistance. **(C)** A picture of the actual unit completely assembled. The yellow arrows indicate the current flow direction.

Define Parameters

```
int analogPin=0;
int PinReading=0;
int V0=5;
int t=0;
int i=1;
float V1=0;
float V2=0;
float R1=117000;
float R2=0;
```

Arduino Setup

```
void setup() {
  Serial.begin(9600);
  Serial.println ("Time/min;Resistance/Ohm");
}
```

Measurements

```
void loop() {
 PinReading=analogRead(analogPin);
 if(i<=61){
  V1=V0*(PinReading/1024.0);
  V2=V0-V1;
  R2=(V2*R1)/V1;

  Serial.print (t);
  Serial.print (";");
  Serial.println(R2);
  delay(60000);
  t=t+1;
  i=i+1;
 }
}
```

Figure 3. The Arduino code to measure TEER every 1 min for 1 hr.

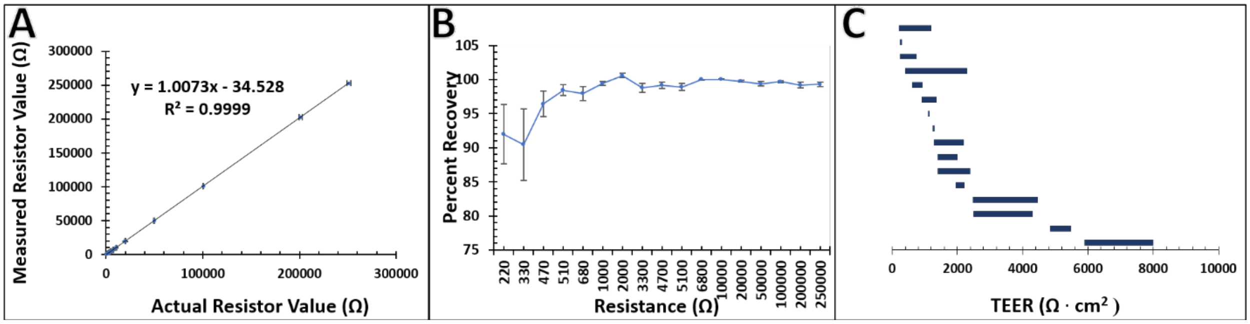

Figure 4. Quantitative characterization of the TEER meter. **(A)** Results of measuring resistors of known resistance values. The slope that is very close to 1, and the small y intercept suggest quantitative recovery of the known resistances. **(B)** Recovery rate of the known resistances measured using our TEER meter. Within the range of 400-20,000 Ω, the measurement variance is < 3%. Because TEER results are typically express by the resistance flux (integrated resistance across the cell monolayer area, $\Omega \cdot cm^2$) and 24 well plate transmembrane inserts (0.33 cm^2) or smaller microfluidic interfaces are usually used, our meter can accurately measure TEER values of (400-20,000 Ω) · 0.33 cm = 132-82500 $\Omega \cdot cm^2$. **(C)** A literature study reveals that most of the reported TEER values of various cell types/culture methods fall between 200 and 8000 $\Omega \cdot cm^2$, which our TEER meter totally covers. The bars are the TEER ranges that the literature reported.

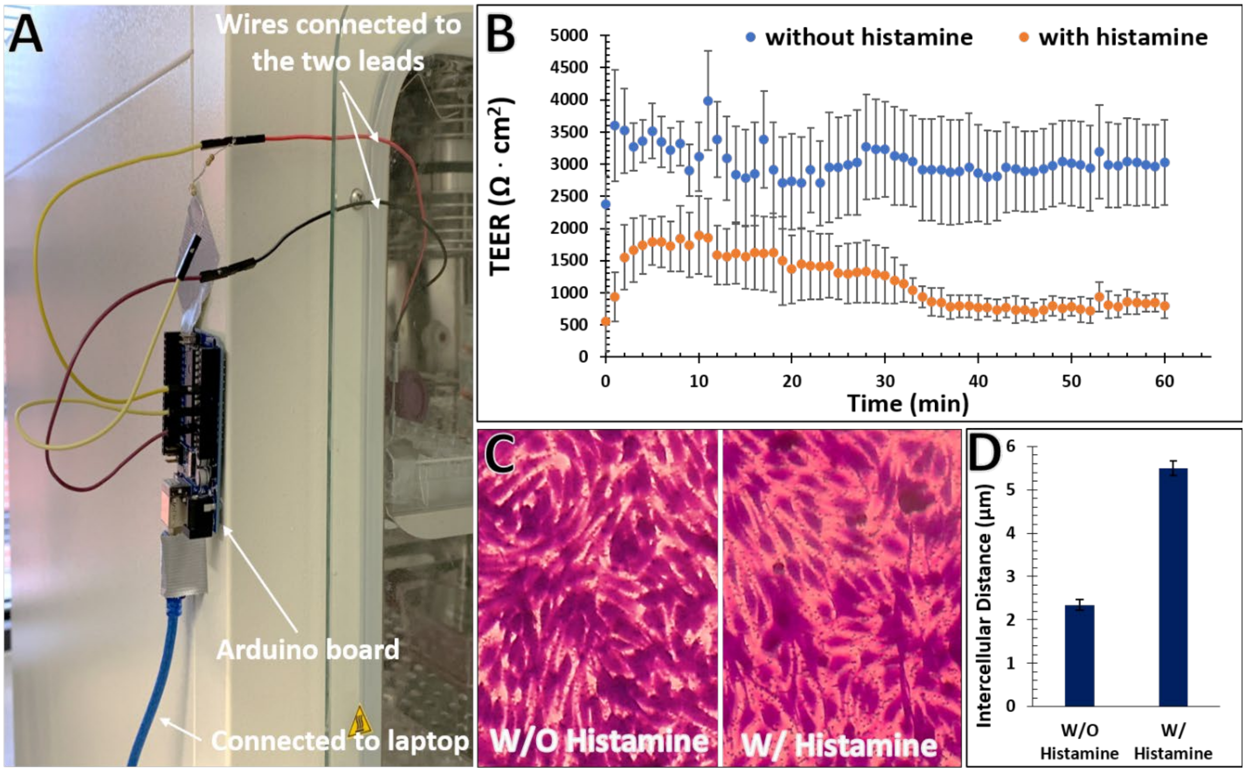

Figure 5. **(A)** The TEER meter can simply be taped on an incubator to measure cells cultured inside. (**B)** TEER measurements across endothelial cells cultured on 24 well-plate membrane inserts with or without histamine treatment. Without histamine, the TEER values are consistent around 3027.5 $\Omega{\cdot}cm^2$. Histamine is known to induce leakage of endothelial monolayers and our meter did detect lower and decreasing TEER values. **(C)** To confirm the TEER measurements, we imaged the cells after the measurement. The cells with histamine appear to show larger intercellular gaps. **(D)** Analyzing the images using ImageJ demonstrate that histamine significantly increased the intercellular gap by ~2 times, which corresponds to the TEER measurement. N=3, $p<0.001$.

Table 1. Estimated costs to make the TEER meter.

Item/Labor	Cost
Arduino UNO board	$12
Arduino software	Free
Jump wires and connectors	$10
Stainless steel wire	$8
Carbon ink	$15
Resistors	$10
Build time	~2 hours

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Supplementary Information

A Low-Cost and Customizable TEER Meter for the Measurements of Cellular Barrier Integrity

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Materials Needed:

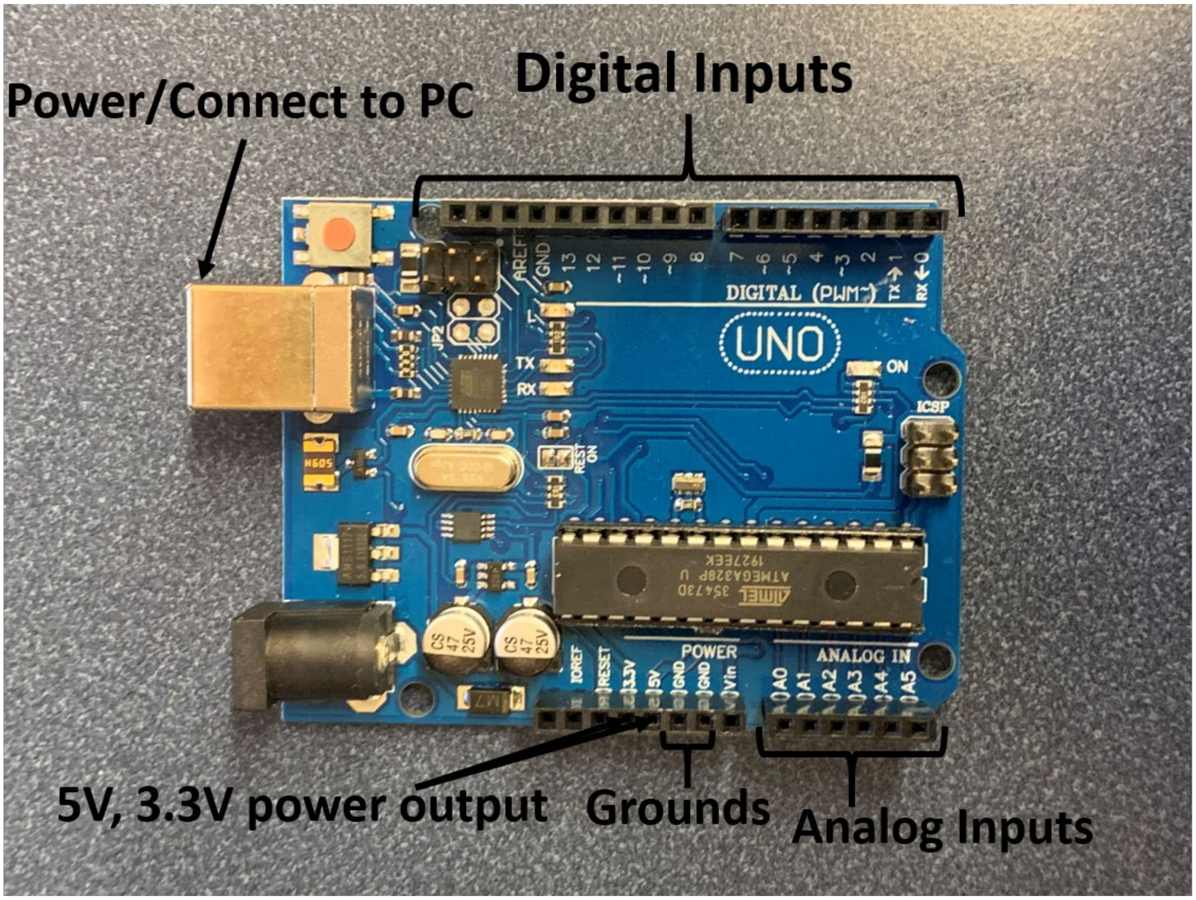

Figure S1. A top down view of the Arduino UNO board used in this experiment. Labelled are the different pin locations on the board. In our experiments, the 5V power output pin, a ground pin and the A0 analog input pin were used. The connection to a computer requires a USB A to B cable.

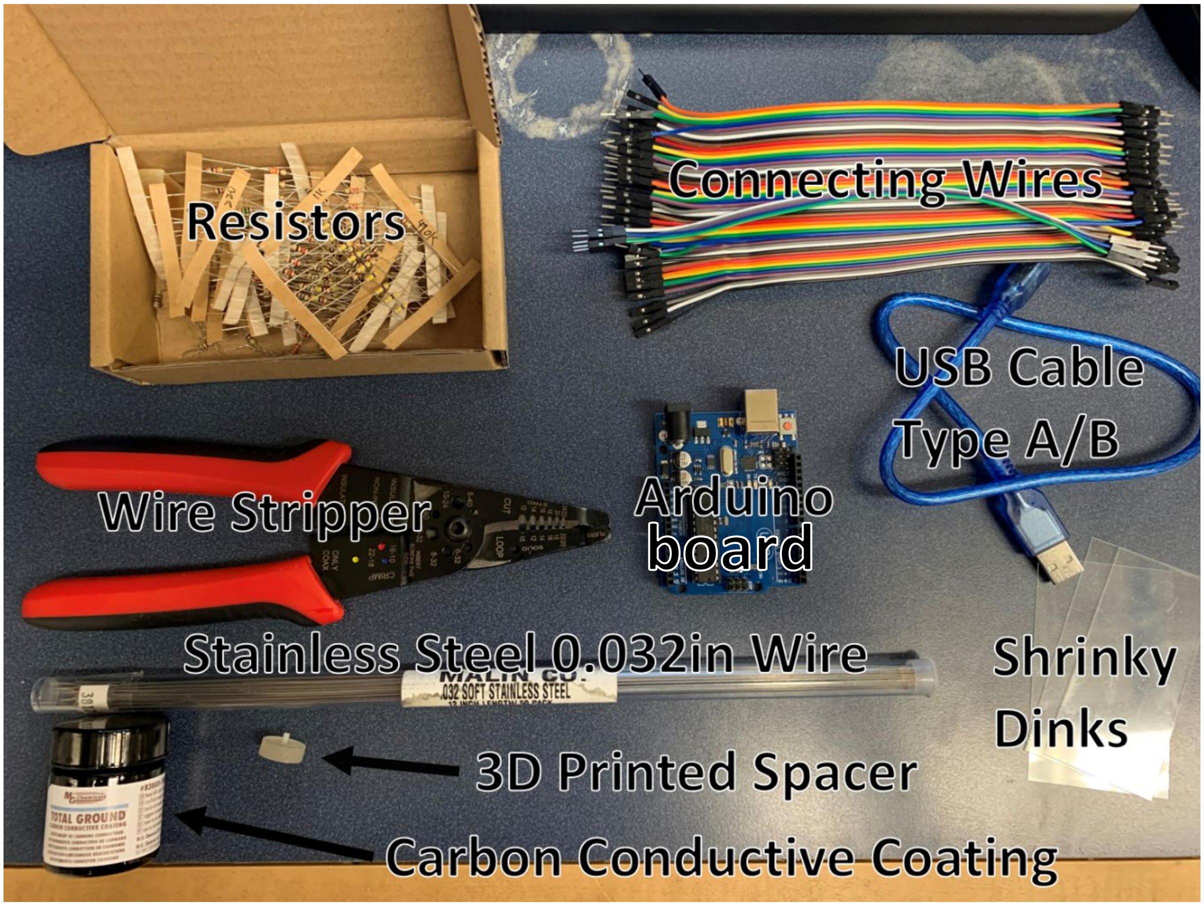

Figure S2. The materials used to fabricate the TEER meter. First the electrode was fabricated. Using the wire strippers, the leads for the electrode were made by cutting the stainless-steel wire into two pieces roughly 15cm long. Then using a cotton swab (not pictured), the carbon conductive coating was mixed until the consistency of the ink was uniform. Then one end of each lead was dipped into the ink and left to dry in air. Then the other side of the lead was inserted through the holes on the 3D printed spacer. The outside lead was measured so that the side with the carbon coating protruded 15mm out and the inner lead measured 12mm. Then the two leads were epoxied onto the 3D printed spacer using the Gorilla Glue brand epoxy (not pictured).

If a 3D printer is not available, another electrode fabrication method was designed. First, a sheet of shrinky dinks (polystyrene sheet) was cut into 3 squares. The 3 squares were placed on top of each other and using a soldering iron (any heat source will work), the edges were heated up so they would fuse together and create a more rigid support for the electrode leads. Then using a ruler, two dots were made on the sheet 5mm apart (the dimensions needed for the leads to correctly fit a porous membrane insert inside a 24 well plate). The remaining piece of the stainless-steel wire that was not apart of the electrode was heated up using a Bunsen burner and pushed through the support structure. Then, each electrode was epoxied onto the shrinky dinks square with the same distance protruding out as mentioned above. This electrode was simple to fabricate and functioned the same as the one made with the 3D printed piece.

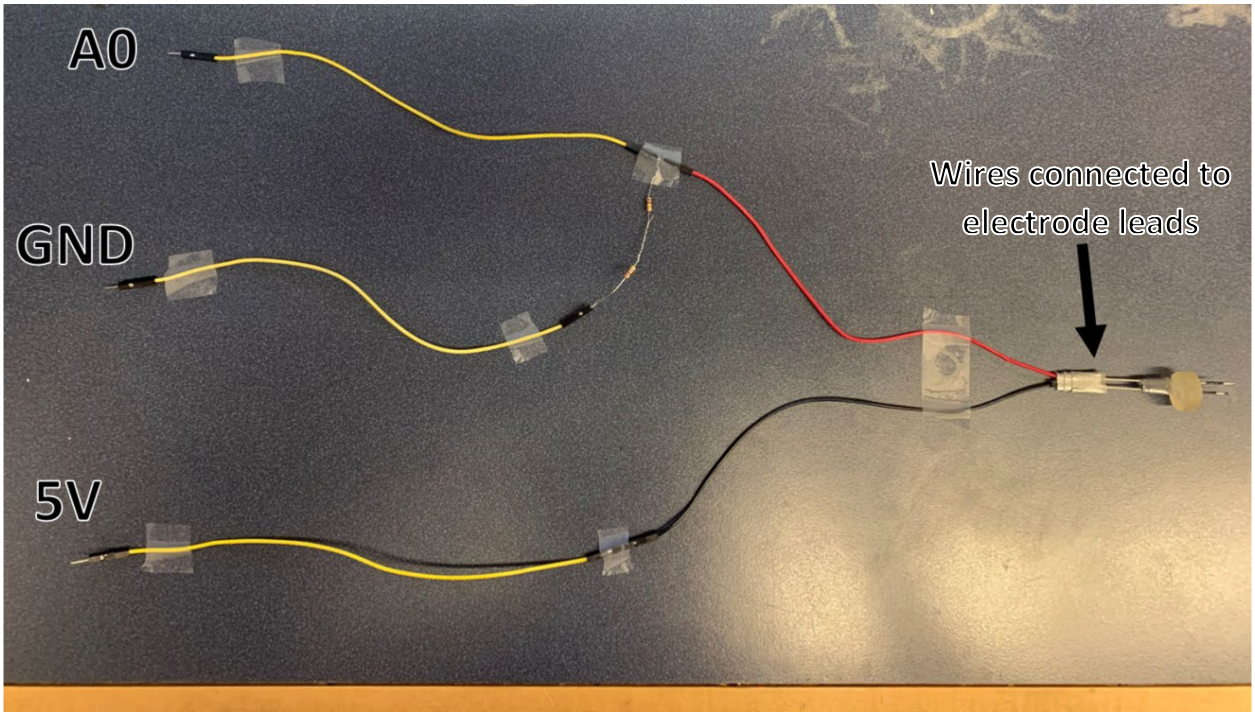

Figure S3. Once the electrode was fabricated the wiring was connected to the electrode. The wiring scheme is shown in the paper in **Fig. 2A**. During the construction of this, the ends of the electrode leads were duct taped together to reduce the chance that the leads would be dislodged from the wiring during the experiment. We did not have a 120kΩ electrode as a part of the resistor kit that was purchased so a 100kΩ resistor and a 20kΩ resistor were wrapped together, and after being tested by a multimeter, the resistance was measured as 117kΩ. One end of the resistor and the male end of the wire coming from the electrode were both inserted into the female input of the wire connecting to pinA0 in order to create the voltage splitting junction. The left side of **Fig. S3** shows the pin that each of the 3 wires connect to on the Arduino board.

Programming the Arduino

```
int analogPin=0; //line 1
int PinReading=0; //line 2
int V0=5; //line 3
int t=0; //line 4
int i=1; //line 5
float V1=0; //line 6
float V2=0;  //line 7
float R1=117000; //line 8
float R2=0; //line 9
//line 10
void setup() { //line 11
  Serial.begin(9600); //line 12
  Serial.println ("Time/min;Resistance/Ohm"); //line 13
} //line 14
//line 15
void loop() { //line 16
 PinReading=analogRead(analogPin); //line 17
 if(i<=61){   //line 18
  V1=V0*(PinReading/1024.0); //line 19
  V2=V0-V1; //line 20
  R2=(V2*R1)/V1; //line 21
//line 22
  Serial.print (t); //line 23
  Serial.print (";"); //line 24
  Serial.println(R2);  //line 25
  delay(60000); //line 26
  t=t+1; //line 27
  i=i+1; //line 28
 } //line 29
} //line 30
```

Fig. S4: The code created to make TEER measurements.

Table S1: The Function of Each Line in the Code

LINE NUMBER	FUNCTION/PURPOSE
1	defines the analog port that data will be collected from as A0
2	defines a variable that will be used to store the voltage times samples at a time point
3	sets a variable representing the input voltage from the Arduino board, which is always 5V
4	defines a timestamp variable that corresponds to every resistance measurement
5	a counting variable that adds one count to the total value, used to terminate the program after a set number of i measurements has occurred
6	the variable that stores the voltage being measured at the analog input A0
7	the variable that stores the voltage after the voltage drop along the sample (R2)
8	the variable of the known resistor constructed in the circuit, R1. In this case it is 117,000 ohms
9	this variable stores the resistance of the sample, measured by the electrode
10	blank
11	void setup is the function that tells the Arduino how it will be running when the program is booted up
12	serial begin tells the Arduino what rate in bits per second (baud) to report to the serial monitor of the Arduino IDL software. 9600 baud is the standard rate so it should not be changed
13	serial print ln prints in the serial monitor the format that the data will be displayed: the timestamps and the resistance separated by a semicolon
14	The bracket ends the void setup function. The parenthesis and semicolons are important syntax in the coding that cannot be left out
15	blank
16	the void loop is the main function of this program. A void loop runs the entire function continuously until a condition is met, which terminates the loop
17	in the first step of the void loop, the analogRead command reads the analogPin variable, which is the voltage at A0, in parts.
18	this is where the conditions of the void loop is set. If the number i is less than or equal to the specified value, then the loop will keep running. Once the value of i is greater than the specified number, the loop ceases to function. In this case, i muct be less than or equal to 61, which if a measurement is recorded every minute plus a t=0 measurement, the loop will continue for an hour and then stop after 60 minutes
19	now that the loop is running, first V1 which is the voltage measured at A0, is converted from parts into a voltage. As stated in the paper, Arduino's standard reporting units are in parts, out of 1024. Therefore V0 needed to be defined as a constant 5V earlier, the signal recorded at A0 needs to be converted from parts to volts, which is a simple unit analysis of input*(5V/1024parts)
20	Then, V2, which is the voltage drop across the sample is defined by subtracting V1 from the 5V power input (V0)
21	Now that we know R1, V1 and V2, the resistance across the sample (R2) is calculated and defined by R2

22	blank
23	Next, since we now know the resistance, a timestamp of the timepoint (in minutes) is displayed in the serial monitor using the serial print function
24	Then, a semicolon is printed to separate the timestamp from the resistance
25	The resistance across the sample is then reported in the serial monitor
26	After the data has been recorded, the void loop is delayed by 1 min. Arduino's delay function counts in milliseconds, so a 1-minute delay is 60,000 milliseconds. This effectively how the sampling rate of the TEER meter is defined and can be changed to whichever increment the user wants
27	Next, a count is added to the timestamp. Again, this can be customized to the user's wish. In this configuration it adds 1 count to the timestamp (for each minute the data is sampled). However if I wanted the report to be measured in seconds as opposed to minutes, the i count would remain the same because we'd be taking the same number of measurements but it would be t=t+60, so the timestamp would appear as: 0, 60, 120 ,...etc.
28	Then a count is added to the total i value. Once this overlaps the defined value of i by the if statement, the loop will no longer run. In this case, when i=62, the loop stops running.
29	This bracket concludes the if portion of the void loop, and until the i value overcomes the set limit, the loop will keep repeating
30	This bracket concludes the entire void loop. A void loop can contain an "else" function, which once the "if" statement concludes, the void loop starts to utilize the "else" command. This program doesn't include that so the program simply stops and will restart again once the serial monitor is closed and reopened

The combination of **Fig. S4** and **Table S1** go into detail about how the code was made. **Fig. S4** is the entire code that was input into the Arduino IDL software, which each line number commented to correspond with a description in **Table S1**. A very important part of the coding are the brackets and the semicolons present throughout the code. They are necessary for the code to function properly; the semicolons serve to end a line and the brackets enclose the different processes of the program. When using this code, if all characters are not inserted correctly, the program will not run properly.

Recording and Saving Data

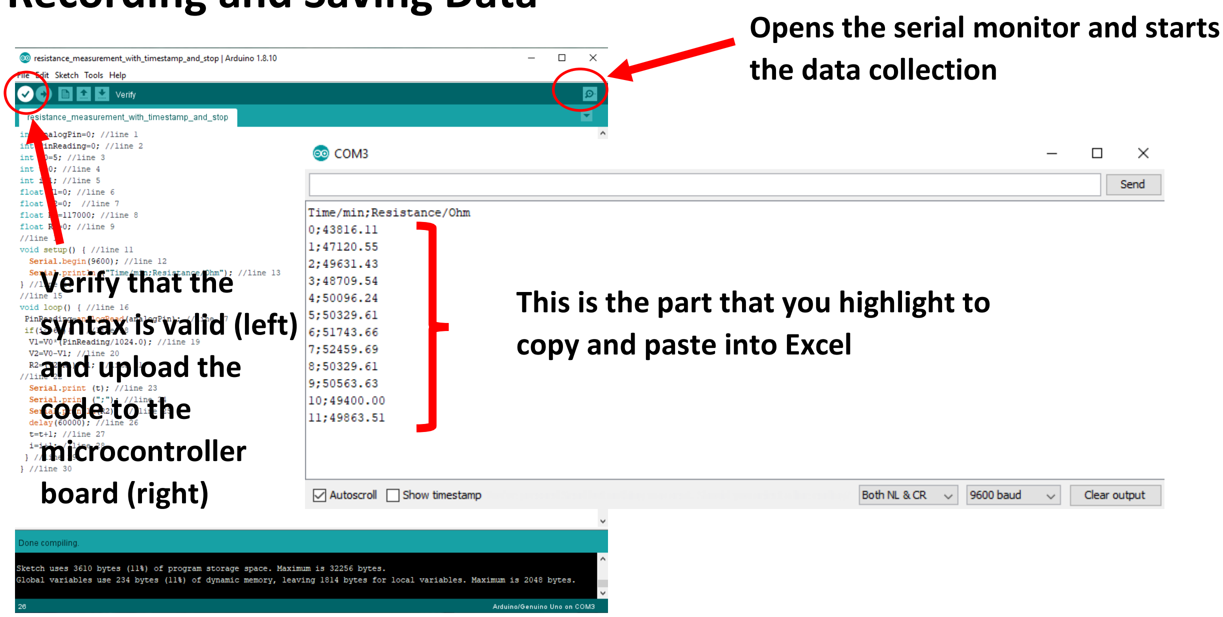

Fig. S5: The Arduino IDL software and the serial monitor window. In order to run the program, first the program needs to be opened in the Arduino IDL software. In order to run a program, the syntax needs to be verified. The IDL software has a built-in function that scans the code for any improperly written code (circled top left) and will tell you what is incorrect so it can be fixed. Then the properly written code can be uploaded to the microcontroller board (circled top left next to the verification function). After the program has been uploaded to the board it doesn't need to be done again unless any edits have been made in the software.

The top right corner of the Arduino software (circled on the right) opens up the serial monitor, which is how the resistance in this program is recorded. Upon clicking the button, the serial monitor opens in a new window and starts to collect data at the specified interval. When the data collection is done, the window will remain open. We transferred the raw data from the serial monitor to Excel directly. In the serial monitor window select all the data and copy it to the clipboard (ctrl+c). Do not highlight the Time/min; Resistance/Ohm text in the selection because it will cause the data to not be pasted (ctrl+v) into Excel.

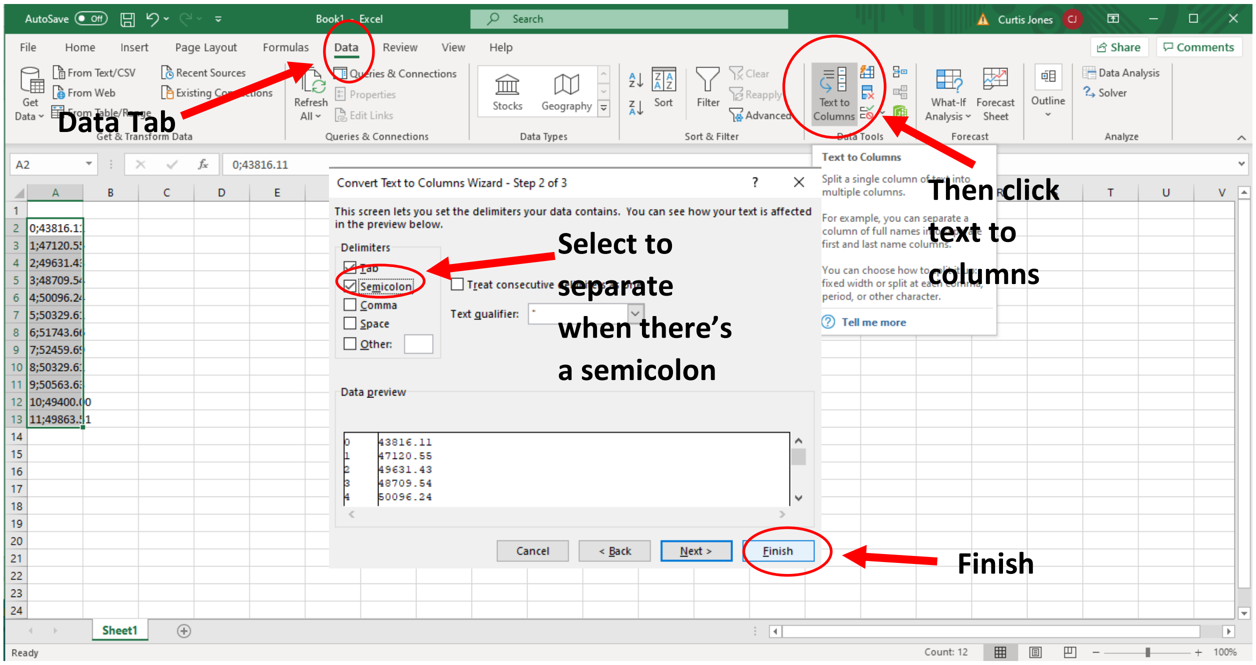

Fig. S7: Transferring the data into Excel. Once the data from the serial monitor on the clipboard has been put into an Excel spreadsheet (ctrl+v) the time and the resistance needs to be separated into two columns. To do this, go to the data tab (circled on the top) in Excel and click on the text to columns button (right). A menu will be brough up to split a set of data into different columns. Click next to go to step 2 of 3 where you select the parameters for splitting into multiple columns (pictured) and click the box that says semicolon. In the Arduino program, we specified the reported measurements to be separated by a semicolon so that when we paste them into excel, they are easily separated into different columns by using this feature. After the semicolon box has been selected, click finish and then the data will be separated into two columns, the left column containing the timestamp and the right with the respective resistance measurement, seen below.