Published in final edited form as:

Lab Chip. 2017 June 27; 17(13): 2264–2271. doi:10.1039/c7lc00155j.

Organs-on-Chips with integrated electrodes for Trans-Epithelial Electrical Resistance (TEER) measurements of human epithelial barrier function

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Abstract

Trans-Epithelial Electrical Resistance (TEER) is broadly used as an experimental readout and a quality control assay for measuring the integrity of epithelial monolayers cultured under static conditions *in vitro*, however, there is no standard methodology for its application to microfluidic Organ-on-a-Chip (Organ Chip) cultures. Here, we describe a new microfluidic Organ Chip design that contains embedded electrodes, and we demonstrate its utility for assessing formation and disruption of barrier function both within a human Lung Airway Chip lined by a fully differentiated mucociliary human airway epithelium and in a human Gut Chip lined by intestinal epithelial cells. These chips with integrated electrodes enable real-time, non-invasive monitoring of TEER and can be applied to measure barrier function in virtually any type of cultured cell.

Graphical abstract

Trans-epithelial electrical resistance (TEER) monitoring of human lung airway epithelium during long term maturation and experimental manipulation in an Organ-on-a-Chip

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Competing Financial Interests

D.E.I. is a founder and holds equity in Emulate Inc., and he chairs its scientific advisory board. R.V. is currently an employee of Emulate Inc.

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Introduction

Organs-on-Chips (Organ Chips) are microfluidic cell culture devices that contain continuously perfused hollow microchannels inhabited by living cells arranged to simulate tissue- and organ-level physiology that are currently being explored as potential replacements for animal testing [1]. One of the main advantages of this type of microphysiological culture system is that it enables high-resolution, real-time imaging analysis of living human cell structure and function in a tissue- and organ-level context *in vitro* [2, 3]. In Organ Chip studies, one of the most useful measures of epithelial or endothelial tissue viability and function is the state of the permeability barrier. Measurement of Trans-Epithelial Electrical Resistance (TEER) is a quick, conventional, and non-invasive assay that is used to evaluate the level of integrity and differentiation of *in vitro* epithelial monolayers in conventional static cultures because the electrical impedance across an epithelium or endothelium is directly related to the formation of robust tight junctions between neighboring cells [4]. While use of TEER measurements of cell cultures has become a standard experimental method to estimate cell monolayer integrity, TEER measurements in Organs on Chips remains technically challenging as no practical and validated approach has been developed. This is because the closed, micrometer-sized, microfluidic channels that support cell growth and differentiation in Organ Chips restrict easy access to the epithelium and make it difficult to carry out TEER measurements. Thus, it is virtually impossible to record changes in permeability continuously using TEER in microfluidic culture systems [5].

Patterned electrodes have been integrated into PDMS microfluidic devices in the past [6], but only a few have been used to monitor epithelial barrier function *in situ* within Organ Chips. This was accomplished previously on-chip by direct insertion of metal wires into pre-molded locations above and below membrane-supported cell monolayers [7–12], repeated insertion of manually manipulated electrodes normally used to measure TEER in Transwell experiments [13], construction of cell culture chambers around large electrodes [14], or integrating glass or polymeric substrates that contain electrodes formed using conventional metal patterning techniques into microfluidic culture devices [15–17]. As a result, the results of TEER studies with cells cultured in Organ Chips presented to date very often suffer from large measurement variability, low sensitivity and they can be highly affected by non uniform cell cultures. Electrode location also can significantly alter TEER readouts in these cultures, although mathematical models have been proposed that can help reduce these

variations [5, 17, 18]. Thus, successful fabrication of a robust on-chip TEER sensing capability would allow performing simple electrochemical measurements to reliably study barrier function. In addition, because this type of method measures electrical impedance, it also could be used to monitor ion channel activity, tissue conductivity, dissolved gases, cell proliferation, migration and many other cell behaviors. In the present study, we therefore set out to develop methods to fabricate Organ Chips with fully integrated electrodes.

To address this ongoing issue, we developed a new 2-channel Organ Chip design with integrated electrodes that enables real-time measurement of TEER across virtually any type of cell monolayer cultured inside the device. Here we show how to fabricate these TEER chips, and as a proof of concept, we demonstrate that they can be used to continuously monitor the formation and disruption of a well-differentiated human lung airway epithelium growing inside a recently described microfluidic human Lung Airway Chip [19]. We also confirmed that similar TEER measurements can be carried out with human intestinal epithelium cultured within the same chips. Thus, these TEER Chips potentially can be used to non-invasively monitor the integrity of virtually any type of cultured tissue or cell monolayer in real-time. It also may provide a convenient quality control assay to assess cell growth and differentiation inside microfluidic cell culture devices.

Material and Methods

Fabrication of TEER Chips

To pattern the electrodes to fit within microfluidic organ chips, polycarbonate (PC) sheets (1 mm thick) were cut into 30 × 40 mm substrates with their protective backing and inlets and outlets drilled as required. After the protective backings were removed, the PC substrates were rinsed with isopropanol, dried in a stream of compressed air and activated in oxygen plasma for 2 minutes (Technics Micro Stripper Series 220, 20 SCCM O₂, 300 mT, 100 W). The electrode patterns were laser cut in silicon-coated paper backing and consisted of two 1 mm wide electrodes separated by 1 mm. The silicon side of the resulting paper shadow masks were gently applied to the activated PC substrates using a homemade alignment jig. These substrates were sequentially coated with 3 nm of titanium and 25 nm of gold in a metal e-beam evaporator (Denton Vacuum LLC, USA). The paper shadow masks were finally gently peeled off the PC substrates.

Polycarbonate and polyacrylic sheets were purchased from McMaster Carr (USA). PDMS Sylgard 184 was obtained from Dow Corning (USA). All chemicals were purchased from Sigma Aldrich (USA). Microfluidic channels were cut in 1 mm and 0.2 mm PDMS films prepared by spin coating onto acrylic discs. The resulting PDMS coated discs were covered with low tack adhesive tape (Magic Tape 3M) to reduce surface contamination during subsequent processing and storage. The PDMS channels were defined using a CO₂ laser using minimal power to limit channel roughness and ashing of the thicker layer. Finally, the patterned PDMS layers were cut to the size of the polycarbonate substrates using sufficient power to cut through both the PDMS layers and the acrylic disks, thereby producing a PDMS channel on an acrylic “stamp”. The protective tape was removed and channels were cleaned with adhesive tape and isopropanol to remove any loosely bound residue. The stamps were finally covered with a new adhesive tape for storage.

The TEER Chip with integrated electrodes was then assembled following a layer-by-layer approach, schematically presented in Figure 1A. PC substrates and PDMS stamps freed of their protective adhesive were activated in an oxygen plasma (20 SCCM O₂, 300 mT, 100 W, 60 seconds) before being immersed for 20 minutes in a 1% aqueous solution of APTES and a 1% aqueous solution of GLYMO respectively. PDMS and PC substrates were rinsed in water and dried in a stream of compressed air before being aligned, brought in contact, and gently pressed together to ensure conformational contact and baked at 60°C overnight. The acrylic backings were gently lifted off and the PC/PDMS assembly and plasma activated together with laser cut porous PET membranes (0.4 µm diameter pores). PC/PDMS and PET membranes were immersed for 20 minutes in a 1% aqueous solution of GLYMO and a 5% aqueous solution of APTES respectively, rinsed in water and dried in a stream of compressed air. The porous PET membrane was finally aligned and brought in contact with a thin PC/PDMS (0.2 mm) layer comprising the basal microfluidic compartment and covered with a thicker PC/PDMS (1mm) layer that formed the apical microfluidic compartment. The assembled microfluidic chips were finally baked at 60°C overnight (Figure 1B). Porous PDMS membranes were prepared and assembled with PC/PDMS fluidic layers as previously described [12] by sequentially exposing the various PDMS surfaces to oxygen plasma (30 seconds, 20 SCCM, 110 mT, 50 W) and bringing them into conformational contact. Finally, devices were cured at 60 °C overnight.

Epithelial cell culture

Our methods for culture and differentiation of primary human airway epithelial cells (hAECs) in 2-channel microfluidic Lung Airway Chips have been described previously [19]. Briefly, hAECs (Epithelix; Switzerland) were expanded in one T75 cm² tissue culture flask until ~70% confluent using Lonza's BEGM growth medium supplemented with growth factors (CC-3170, Lonza, USA). The porous PET membrane separating the upper and lower microchannels was coated with Type I Collagen (Purecoll, Advanced Biomatrix, USA) and then hAECs were seeded (4.5×10^6 cells/mL) on its upper surface and incubated at 37°C under static conditions to promote cell attachment. Medium was replaced daily with fresh medium for 6 days until the cells become fully confluent and then an air-liquid interface (ALI) was generated to trigger mucociliary differentiation by removing medium from the upper channel. The epithelium was fed by continuously perfusing (60 µL/hr) the lower channel with medium for the duration of the experiment until full differentiation was attained (~3 to 4 weeks). Healthy differentiated epithelium was maintained for 65 days, and the viability and quality of the epithelial cultures were assessed by monitoring epithelium morphology and integrity, cilia beating and presence of mucus secretion by phase contrast microscopy, as previously described [19].

In some studies, human Caco2 intestinal epithelial cells were cultured within these TEER chips using previously described culture methods [12]. In brief, the TEER devices were exposed to oxygen plasma for 30 seconds at a power of 50W using a PE-100 plasma sterilizer (Plasma Etch, Inc. NV, USA), and then treated with 1% (3-aminopropyl)-trimethoxysilane (APTMS; Sigma) in 100% anhydrous ethanol for 10 min at room temperature. After subsequent rinsing with 70% and 100% ethanol washes, the devices were baked at 80°C for 2 hours. The insides of the channels were then coated with rat type I

collagen (100 µg/mL; Corning) in the presence of Matrigel (10 µg/mL) at 37°C overnight in a 5% CO₂ incubator, followed by rinsing with DMEM culture medium. The intestinal epithelial cells were seeded (1×10⁶ cells/mL) on the PDMS membrane and incubated at 37°C for 3 days under static conditions to promote cell attachment before initiating studies and maintained under flow (60 µL/hour) thereafter.

TEER measurements

A PGstat128N from Metrohm Autolab BV (The Netherlands) was used to record impedance spectra. Four-point impedance measurements were taken periodically over a period of 65 days using a PGStat12/FRA (Autolab). Prior to initiating TEER measurements with cells cultured in Organ Chips, 50µL of warm DMEM was gently introduced through the apical compartment to wash away excess mucus. Following the washing step, 50µL of warm DMEM was introduced in the apical compartment and left to equilibrate at 37°C for 10 minutes before carrying out measurements. After measurement, the apical medium was again removed to restore ALI. TEER measurements were performed at room temperature for a maximum of 2 minutes, which did not affect culture quality. In some studies, EGTA (2mM) was introduced in the apical channel, followed by the basal channel 10 minutes later to disrupt tight junctions, and chips were incubated at 37°C for 150 minutes. TEER values were measured every 10 minutes for 1h and then every 30 min thereafter at room temperature. Cells were immediately placed back in incubator immediately after each measurement.

At high frequency (>10KHz), the impedance curves are mostly characterized by the solution resistance, whereas TEER dominates the signal at lower frequency (<100Hz); capacitance is extracted from impedance data in the intermediate range (100Hz-10KHz). This approach facilitates interpretation of TEER data as the background impedance of the system is automatically subtracted by the model. Several models were tested and assessed based on both the goodness-of-fit (χ^2) criteria and their ability to provide a useful understanding of the underlying biology. The selected model, which consists of a resistor R_{SOL} in series with another resistor R_{TEER} and a constant phase element (CPE), fitted all of the data well ($\chi^2 < 0.01$). More complex models with better χ^2 were found to not always be able to fit all data sets or were difficult to interpret. CPEs are not typically used in electrophysiology to model cell capacitance, but they have been shown to better fit the measured impedance of many cells [17]. We also found this element to be particularly useful to model the early stages of cell growth (<6 days). The mathematical expression of a CPE impedance is:

$$Z_{CPE} = \frac{1}{Y_o(j\omega)^n} \quad \text{Equation 1}$$

in which the CPE's impedance is expressed as a function of the system's admittance Y_o , and an exponent n equaling 1 or 0 for an ideal capacitor or an ideal resistor, respectively. Values for R_{TEER} , Y_o and n were estimated by modeling the experimental data using the equivalent circuit presented in Fig. 2B and using equation 2 to calculate the capacitance of the cell layer C_{cell} expressed in Farad (F).

$$C_{cell} = \frac{(Y_0 \times R_{TEER})^{\frac{1}{n}}}{R_{TEER}} \quad \text{Equation 2}$$

Statistical analysis

All results are expressed as mean $\pm$ standard error. For the statistical evaluation of quantified data, a paired t-test analysis was performed using GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego CA). Differences were considered statistically significant when $p < 0.05$.

Results and Discussion

Integrated TEER chip fabrication

We designed an integrated TEER chip (Fig. 1A and 2A) that includes four electrodes (2 above the upper channel and 2 below the lower channel) integrated into a microfluidic device containing upper and lower channels separated by a porous (0.4 μm diameter) PET membrane or PDMS membrane, similar to those that we previously used to create a human Lung Small Airway Chip [19] and human Gut Chip [12], respectively. The electrodes were patterned on polycarbonate (PC) substrates using a laser patterned, silicon-coated, backing paper, shadow mask. The electrodes were 1 mm wide spaced by 1 mm, but well defined patterns as small as 0.3 mm can easily be achieved using this simple technique. Shadow masks were carefully peeled off their substrates and put in contact with plasma-activated PC sheets. A thin 3 nm titanium adhesive layer was first evaporated onto the masked PC, followed by 25 nm of gold. The resulting pattern was very stable to isopropanol or ethanol rinse, as well as sonication for 5 minutes in buffer, and it was even difficult to remove using finger pressure. Importantly, in addition to their stability, these electrodes proved to be transparent which allowed for qualitatively assessing cell cultures using optical microscopy. Inlets and outlets were drilled on the top parts where required.

We chose PC as a base substrate for its high optical clarity, cell culture biocompatibility, ease of machining, compatibility with metal deposition processes and ease of chemical surface modification via silane chemistry to promote bonding to other polymer surfaces. Aminopropyl triethoxysilane (APTES) can readily interact at the surface of plasma activated PC through the formation of stable amide bonds between the acid groups generated during plasma treatment and the amine functionality of APTES [20, 21]. While bonding of plasma activated PDMS to the silanol groups introduced at the PC surface via APTES treatment has been reported [22], we found that prior introduction of epoxy moieties at the PDMS surface using GLYMO resulted in improved bonding [23–25] and long term resistance to hydrolytic cleavage. The porous PET membrane was then bonded to the open face of the PDMS layers using the same strategy, first modifying the PET surface with APTES and the PDMS with GLYMO before assembling the final device (Fig. 1B) and overnight curing at 60°C. This resulted in a very stable bonding. As previously reported [24, 25], the APTES/GLYMO bonding can occur very rapidly at room temperature, however we found that overnight at 60°C produce more robust devices. The difference in thermal expansion coefficient between

PDMS and PET did not result in the deformation of the membrane and it was found beneficial to flattening the membrane in the assembled device. The porous PDMS membrane was bonded to the PC/PDMS fluidic layers using oxygen plasma and did not require any additional treatment.

Importantly, while glass also may be used to create microfluidic cell culture devices with integrated electrodes, PC has major advantages in terms of its ease of machining. For example, electrodes can be easily patterned on PC or PET using techniques, such as conventional lithography and metal patterning techniques, as well as roll-to-roll laser ablation, which can considerably lower the cost of the final devices. Drilling and cutting glass also requires instruments that are not always available in research laboratories. Another advantage of our technique is that our chips can be assembled without requiring additional layers for bonding, or to provide fluidic access.

TEER measurements in Organ Chips

Before initiating studies with Organ Chips, we carried out control experiments measuring impedance of aqueous solutions of 1, 10, 100 mM NaCl as well as the DMEM culture medium we use for our Organ Chip experiments (which contains 109 mM NaCl, as well as various other components). These studies confirmed that this impedance sensor is sensitive to changes in conductivity (Supplementary Figure S1). As expected, the impedance response recorded for DMEM was slightly lower than that of 100 mM NaCl as it holds a slightly higher conductivity. TEER was then measured in 4 different Organ Chips, each containing a well differentiated, mucociliated, human small airway epithelium cultured in the upper channel at an air liquid interface (ALI) while the cells were fed by flowing (60 μ L/hr) growth medium through the lower channel, as described previously [19]. All 4 Lung Airway Chips were maintained for 62 days in culture and 56 days at ALI, without any evidence of cell toxicity from the presence of the gold and titanium layers. TEER measurements were carried out using a 4-points impedance measurement (Fig. 2A) over the frequency range 100 kHz to 10Hz and the impedance curves fitted to an equivalent electric model (Fig. 2B). This simple model allows extracting both TEER and cell capacitance values very effectively by factoring in the conductivity of the solution in every measurement (see Methods for Details).

TEER measurements were recorded at days 1, 4, 6, 17, 22, 46 and 62 post seeding, and impedance and capacitance values were determined for each time point (Fig. 3). Analysis of these data revealed that during the first 6 days when cells were cultured submerged in medium (before ALI), TEER values oscillated between an average of 200 Ohms (Ω) (day 4) and 500 Ω (day 6), consistent with the progressive establishment of a tight monolayer of submerged airway epithelial cells. Creation of an ALI by removing the apical growth medium and flowing differentiation medium in the bottom compartment resulted in a steady increase of TEER from 500 Ω at day 6 (0 day at ALI) up to an average 1700 Ω at day 62 (56 days at ALI). The capacitance of the cell layer followed a similar trend, stabilizing after day 22 to an average value of 220 nF. In addition, the value of n (see Equation 1 in Methods) extracted from modelling the impedance response to the equivalent circuit described in Fig. 2B stayed under 0.5 over the course of the 6 first days of culture (i.e., until the cultured cells reached confluence). Thereafter, n remained greater than 0.9, which can be associated with

the formation of mature cell monolayer, behaving more closely to an ideal capacitor. At those time points, substituting the CPE for a capacitor in the model did not result in significant changes to the value R_{TEER} , R_{SOL} and χ^2 . Importantly, the 10 μ A excitation current used for these measurements did not produce any detectable changes in cell behavior over 2 months of culture.

It is important to note that we present results here as Ω , rather than $\Omega \cdot \text{cm}^2$ (which normalizes TEER as a resistance dependent on the surface area of the cultured tissue being analyzed), because while our TEER chip measures impedance of the culture area directly above and between the electrodes, it also is likely influenced by indeterminate regions of the cell culture outside the edges of the electrodes. While past TEER studies on Transwell inserts and organs-on-chips normalized their results for total tissue culture area and presented them as $\Omega \cdot \text{cm}^2$, most of those studies used hand-manipulated electrodes that can provide misleading results based on their location or the stability of their position. There also was no evidence that the field was homogeneous across the entire tissue culture surface area in those studies, and so the biological significance of the $\Omega \cdot \text{cm}^2$ results they presented remain unclear. Thus, while presenting results in Ω might seem to be a limitation, the major advantage of our method is that the results obtained from one chip are directly comparable to all others obtained with the same TEER chip design because of the robustness of the electrode set-up. However, if investigators who use these TEER chips in the future want to compare their results with those obtained in past studies, they can estimate the value in $\Omega \cdot \text{cm}^2$ by multiplying the results they obtain by 0.03 cm^2 (i.e., the culture surface area above and between the electrodes in our TEER chips), as long as they understand the caveat that the actual surface area may be slightly larger.

To explore whether our TEER chip design can be generalized to other cell types, we cultured human intestinal epithelium for 12 days within the same device, except that it contained a porous PDMS membrane similar to that used in a previously described human Gut Chip [12]. In these studies, the impedance reached a stable plateau in the range 0.5 Hz to 100 Hz, although some instabilities were observed at lower frequencies, below 0.5 Hz (Figure 4). Thus, these results show that the electrical double layer does not interfere with our measurements in the frequency range studied, and they confirm that the method can be used with other types of epithelium.

To explore the dynamic measurement capabilities of the TEER Chip, we then measured impedance and capacitance within the intestinal epithelium in the TEER chip beginning 2 hours after seeding the cells and every day thereafter for 12 days in culture (Fig. 5A). These studies revealed that impedance values rapidly increased to a plateau of $4046 \pm 210 \Omega$ by culture day 3, while the capacitance stabilized at $182 \pm 17 \text{ nF}$, which corresponds to the rapid formation of a stable intestinal epithelial monolayer. The chips were then placed under flow beginning on day 4, which initiated further differentiation and promoted the formation of three dimensional intestinal villi-like structures, as previously described [12]. Capacitance increased very rapidly thereafter to plateau at $1033 \pm 269 \text{ nF}$ by day 11, and this was associated with an increase in tissue surface area during differentiation due to villi formation, while impedance decreased steadily to stabilize to $867 \pm 131 \Omega$ (Fig. 5A).

Capacitance correlates very well with cell membrane surface area and correlation factors have been proposed [26, 27]; thus, capacitance potentially can be used to correct impedance values for total cell membrane area. When we normalized impedance based on the measured capacitance values, we found that the corrected impedance values increased steadily during the first 4 days of culture, and then remained at a steady plateau value thereafter (Fig. 5B).

To verify that the increases in TEER values we measured were specifically related to the presence of tight junctions, we treated the intestinal epithelium both apically and basally at day 13 with or without EGTA (5 mM) for 2 hours during which the impedance of the cultures was monitored regularly (Fig. 5C). Because EGTA is a strong Ca^{2+} chelator, it will affect adherens junctions as well as tight junctions, although deficiency in Ca^{2+} alone is not normally sufficient to induce these effects. When exposed to EGTA, the impedance of the epithelium dropped by 50% within the first 20 minutes and reached a maximum reduction of 65% by 1 hour (Fig. 5C), which is consistent with previous reports using conventional TEER measurements in static cultures [28]. In contrast, control chips maintained near constant impedance values over the same time course (Fig. 5C). EGTA treatment also was accompanied by a loss of distinct boundaries around the villus-like epithelial structures when contrasted with untreated Gut Chips (Fig. 5D), which is consistent with disruption of tight junctions and loss of epithelial organization. Importantly, by washing out the EGTA after 2 hours, and culturing the intestinal epithelium in control medium overnight, we could confirm the reversibility of this effect by measuring impedance using the TEER chip during the recovery period and demonstrating restoration of impedance values by the end of the culture period that were indistinguishable from those measured at the start of the experiment (Fig. 5C). Restoration of normal TEER values also was accompanied by reappearance of more well defined villus-like structures, when analyzed by phase contrast microscopy (Fig. 5D). Control experiments carried out with EGTA and DMEM medium alone without cells also confirmed that EGTA has only a minimal direct effect (5.36 Ω increase in impedance at 12 Hz) on background impedance measurements (Supplementary Figure S2).

Similar effects were also observed in human pulmonary epithelium cultured in the Lung Airway Chip 65 days post ALI. EGTA treatment (2 mM) resulted in a rapid drop in impedance values averaging 1800 Ω to 500 Ω within 150 minutes after which impedance levels plateaued (Supplementary Figure S3 A, B). The presence of EGTA in the apical channel resulted in a very limited drop in TEER believed to be the consequence of slow EGTA diffusion through the mucus layer [19], whereas subsequent introduction of EGTA via the basal channel resulted in a more severe effect on the cell-cell junctions. These observations are in agreement with the mode of action of EGTA, which disturbs epithelial cell-cell adhesions, but does not significantly affect epithelial-matrix attachment or cell shape [29, 30]. This also was supported by phase contrast microscopic analysis that revealed EGTA treatment results in opening of the junctions between neighboring epithelial cells without causing obvious cell detachment, rounding or monolayer disruption (Supplementary Figure S3 C, D).

Conclusion

The full integration of electrodes within Organ Chip microfluidic culture devices remains challenging. We developed a simple layer-by-layer fabrication process that enables assembly of complex microfluidic Organ Chips with integrated, semi-transparent, sensing electrodes to measure both TEER and cell layer capacitance using 4-points impedance measurements at varying frequencies. Using this approach, we were able to follow the differentiation of human primary small airway epithelial cells under ALI culture conditions and human intestinal epithelial cells covered by flowing medium on-chip, as well as the disruption of cell-cell junctions and accompanying drop in TEER levels upon exposure to the chelating agent EGTA. While we feel our system provided sufficient sensitivity, the location, dimensions and design of the electrodes can be further modified to optimize excitation of the cell culture area and measurement of the electrical potential across the tissue barriers depending on the Organ Chip designs utilized for culture. Nonetheless, our newly developed TEER-chip platform enables real-time measurements of barrier function that can be used to assess Organ Chip viability and changes in function in response to basal culture conditions, as well as drugs, toxins, inflammatory mediators, or other relevant external stimuli. This TEER chip technology also may open up new applications, including measurement of short circuit current, or action potentials of electrically active cells for electrophysiology studies, which are currently lacking in the majority of microphysiological systems, thus expanding the breadth of use of Organ Chips.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was conducted with support from DARPA (#HHSF223201310079) and the Wyss Institute for Biologically Inspired Engineering at Harvard University. We thank Andres Rodriguez for his assistance with graphic design.

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Figure 1.

A. Exploded CAD model of the TEER-chip. Gold electrodes are patterned onto polycarbonate substrates. Laser cut PDMS layers and PET membrane are assembled using silane-based surface modification to irreversibly bond together. **B.** Photograph of the assembled TEER-chip, dimensions are 25×40 mm.

Figure 2.

A. Schematic view of the TEER-chip and 4-point impedance measurement chosen to measure TEER and capacitance. A small current of 10 μ A of varying frequency is applied between two electrodes (I_{excite}) located on each side of the cell culture, and the drop in potential between the second set of electrodes measured (V_{meas}). **B.** Example of impedance spectra recorded before ALI and after full differentiation at ALI. Lines are fitted data based on the equivalent electronic circuit used for modelling.

Figure 3. Graph showing the TEER and capacitance data recorded over the course of differentiation of the human primary airway epithelial cells. ALI = time of initiation of the air liquid interface (n=4 individual chips).

Figure 4.
Impedance data for human Caco2 intestinal epithelial cells cultured in our TEER chip and recorded in the frequency range 0.1Hz to 100KHz.

Figure 5.

A. Overlay of impedance and capacitance values recorded during growth of intestinal epithelial cells and formation of an epithelial monolayer on-chip (n=5 chips). **B.** Plot of impedance values corrected based on measures of cell capacitance from **A**, which provide an estimate of total cell membrane surface area. **C.** Overlay of impedance recorded during exposure of the TEER chip lined by intestinal epithelium to 5 mM EGTA (black circles; n=3 chips/condition) at different time points after treatment (the 24h recovery period is highlighted in gray). TEER values of control chips cultured in parallel without EGTA were kept was determined at the same time points (open circles; n=2 chips). All TEER values measured for epithelium exposed to EGTA were significantly lower than control values ($p < 0.0007$) at all time points except for the initial and final time points. **D.** Phase contrast images of differentiated human intestine epithelial cells treated without (top) or with (bottom) 5 mM EGTA (bar, 100 μm).