

Supporting Information for:

Phase angle spectroscopy on transparent conducting polymer electrodes for real-time measurement of epithelial barrier integrity

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1. Materials and Methods

2. Supplementary Figure S1

Supplementary Table S1

Supplementary Figure S2

Supplementary Figure S3

1. Supplementary Materials and Methods

Electrochemical and optical characterization

Electropolymerization: PEDOT:PSS was electrodeposited on 2.5 x 3.0 cm Indium tin oxide (ITO) coated PET ($60 \Omega \text{ sq}^{-1}$) (Sigma-Aldrich). Deposition at 0.5 mA for 1200 s with 0.29 mC mm^{-2} was performed using a Gamry Reference 600 potentiostat in a three-electrode setup. Electrodes were immersed in 0.01 M 3,4-ethylenedioxythiophene (EDOT) (Sigma-Aldrich) and 5 mg/ml poly(sodium 4-styrenesulfonate) (PSS) (Sigma-Aldrich) in ultrapure water (**Figure S1a**).

Absorbance: Absorbance of the material was measured at 230-1000 nm. ITO and PEDOT:PSS surfaces were examined in unbiased state, as well as the oxidized and reduced states, the latter achieved by applying a potential of +1.5 V (oxidation) or -1.5 V (reduction), (**Figure S1b**).

Sheet Resistance R_{sh} : R_{sh} was determined using a 4-point probe (Guardian) by passing a calibrated constant current of $45.3 \mu\text{A}$ through the two outer tips and measuring the voltage between the two inner tips (mean \pm sd, $n = 6$), (**Figure S1c**).

Wettability: The contact angle θ of the material was determined using a goniometer (KSV, Finland). A drop of $5 \mu\text{l}$ Milli-Q water was automatically placed on the polymer film and the angle between the surface of the drop and the polymer film was measured (mean \pm sd, $n = 6$), (**Figure S1d**).

Impedance Spectroscopy & Cyclic Voltammetry: Potentiostatic electrochemical impedance spectroscopy and cyclic voltammetry were performed using a Gamry Reference 600 potentiostat with a standard three-electrode setup with a platinum counter electrode and a Ag/AgCl reference electrode in phosphate buffered saline (PBS) (Medicago. For impedance spectroscopy a sinusoidal AC potential with 5 mV (RMS) was applied in a frequency range between 10^{-1} - 10^5 Hz – 100 kHz. Impedance and phase angle were recorded for each frequency step (mean \pm sd, $n = 3$), (**Figure S1e**). For cyclic voltammetry the potential was swept between -1.0 V and +1.0 V with 0.1 V s^{-1} scan rate, and the current was recorded for four voltage cycles. The I-U plot is displayed for cycles 2 – 4 (mean \pm sd, $n = 3$), (**Figure S1f**).

Equivalent circuit modelling

EIS data on the 2-electrode PAS device were recorded for 60 hours with MDCK cells growing on the device as well as with PBS and fully supplemented Dulbecco's modified Eagles medium (DMEM) as control. The obtained data were fitted to established equivalent circuit models using the Levenberg-Marquardt method with the Gamry EChem Analyst Software (Gamry).

The equivalent circuit parameters were chosen according to the qualitative shape of the Nyquist plot in EIS measurements. The plot representing PEDOT:PSS coated ITO in supplemented cell culture medium, shows a high frequency semi-circle, which describes a first-order kinetic system with a single time constant, $\tau = RC$, representing the capacity of the ionic double layer at the electrode-electrolyte interface, C_{dl} , and the charge-transfer resistance R_{ct} (**Fig. 2c**). The frequency at the apex of the semi-circle ω_c can be used to determine R_{ct} and C_{dl} with $\omega_c = (R_{ct}C_{dl})^{-1}$. Characteristically, the diameter of the semi-

circle determines R_{ct} . The intersection of the semi-circle with the real axis at lower resistance corresponds to the value of a serial resistance accounting for the electrical resistivity of electrode material and electrolyte, which was modelled by the solution resistance R_s . An inclined line attached to the semi-circle indicates the presence of diffusion-controlled processes. Thus, a constant phase element (CPE) was added to the model to account for the increased porosity of the PEDOT:PSS electrode. To model increased, almost purely capacitive behaviour of the cell monolayer on the electrode surface, indicated by a capacitive line in the Nyquist plot of the cell monolayer grown on the PEDOT:PSS electrode, an additional RC element with a cell resistance R_c and a cell capacitance C_c was used (**Fig. 2e**).¹¹ Fits with $\chi^2 < 0.001$ were obtained for both cases and a detailed evaluation of equivalent circuit elements is included in the Supplementary information. The fitting parameters are listed in **Table S1**.

Immunofluorescence staining & confocal laser scanning microscopy

Following 24, 48 and 72 h incubation, cells were gently washed in PBS (Medicago), fixed 5 min in 4 % paraformaldehyde (PFA) in PBS at room temperature, then rinsed in PBS. Blocking and permeabilization was achieved by incubation in 5% bovine serum albumin (Sigma-Aldrich) and 0.1% saponin (Sigma-Aldrich) in PBS for 30 min at room temperature. The cytoskeleton was stained by FITC-phalloidin (Life Technologies) diluted 1:1000 in blocking/permeabilization buffer for 1 h at room temperature in humidified atmosphere together with the nucleic counterstain Hoechst 33342 (Life Technologies) diluted 1:1000. The tight junction protein ZO-1 was stained with the rabbit anti-ZO-1 polyclonal antibody (Life Technologies) diluted 1:50 in blocking/permeabilization buffer, and incubated overnight at 4°C in humidified atmosphere. The secondary antibody anti-rabbit-Cy3 (Jackson Laboratories, diluted 1:400 in blocking/permeabilization buffer) was applied together with Hoechst 33342 (Life Technologies) nucleic counterstain (diluted 1:1000) and incubated for 1 h at room temperature. Following gentle rinse in PBS, cells were mounted using Prolong Gold mounting medium (Life technologies). Confocal laser scanning microscopy was performed using an Olympus FV1000 confocal microscope. Circa 15 stacks were obtained with z-steps of 1 μm , z-projections were obtained using ImageJ image processing software (NIH, USA), (**Figure 3d**). Cell numbers were estimated by counting stained nuclei from confocal stacks setting an automatic threshold in the blue channel and analyse particles with size 10 - 400 μm^2 and 0.05 - 1.00 circularity (ImageJ 1.46, NIH, USA) (**Figure 3c**).

Cell culture

Madin-Darby Canine Kidney (MDCK) epithelial cells (ATCC, no. CCL-34) were propagated in DMEM (Sigma-Aldrich) supplemented with 10% FBS (Gibco), 1 % GlutaMAX™ (Gibco) and 1 % MEM Non-essential Amino Acid Solution (Sigma-Aldrich) without addition of any antibiotics. Cells were detached from the cell culture flask with Trypsin–EDTA (Gibco) (0.5 g/l trypsin, 0.2 g/l EDTA in PBS), washed and re-suspended in fully supplemented DMEM (as described above) to a concentration of 2.5×10^5 cells per ml. Aliquots of 1 ml cell suspension were added into functionalized cell culture dishes to an initial density of 2.5×10^5 cells per dish. Cells were incubated in a humidified, 37°C cell incubator with 5% CO_2 .

PAS sensing

For PAS sensing on the transparent, conducting polymer based 2-electrode device a sinusoidal AC potential with 5 mV (rms) was applied between the two electrodes while the current was measured. The impedance Z and phase angle ϕ were determined from the amplitude and phase delay of the current signal with regards to the sourced AC potential. Full spectra PAS was performed in the frequency range of 10^{-1} - 10^5 Hz with 10 data points per decade. Recording of a single full spectrum took in average 4.37 min to complete. Partial spectra PAS for increased time resolution was performed in the frequency range of 10^3 - 10^5 Hz with recording of a single partial spectrum took in average 33.2 s to complete. To monitor the growth of an epithelial cell layer on the device, the potentiostats were programmed to record full impedance spectra for 60 h with 1 h delay between spectra (**Figure 3**). As control, fully supplemented cell culture medium was used and the same measurement regime was followed (**Figure S2**). For experiments investigating disruption of epithelial barrier integrity by ionomycin, MDCK cells were cultivated on the PAS sensing device for 4 days. After reaching confluency full spectra PAS was performed to establish a baseline. After 30 min of full spectra PAS recording, cells were treated with 20 μ l ionomycin (1 mg ml⁻¹ in dimethylsulfoxid (DMSO)) from *Streptomyces conglobatus* (Sigma-Aldrich) or equal volumes DMSO as control. Full impedance spectra were recorded for 90 min with 5 s delay between spectra (**Figure S3**). For increased time resolution, partial PAS spectra were recorded for 90 min with 1 s delay between spectra.

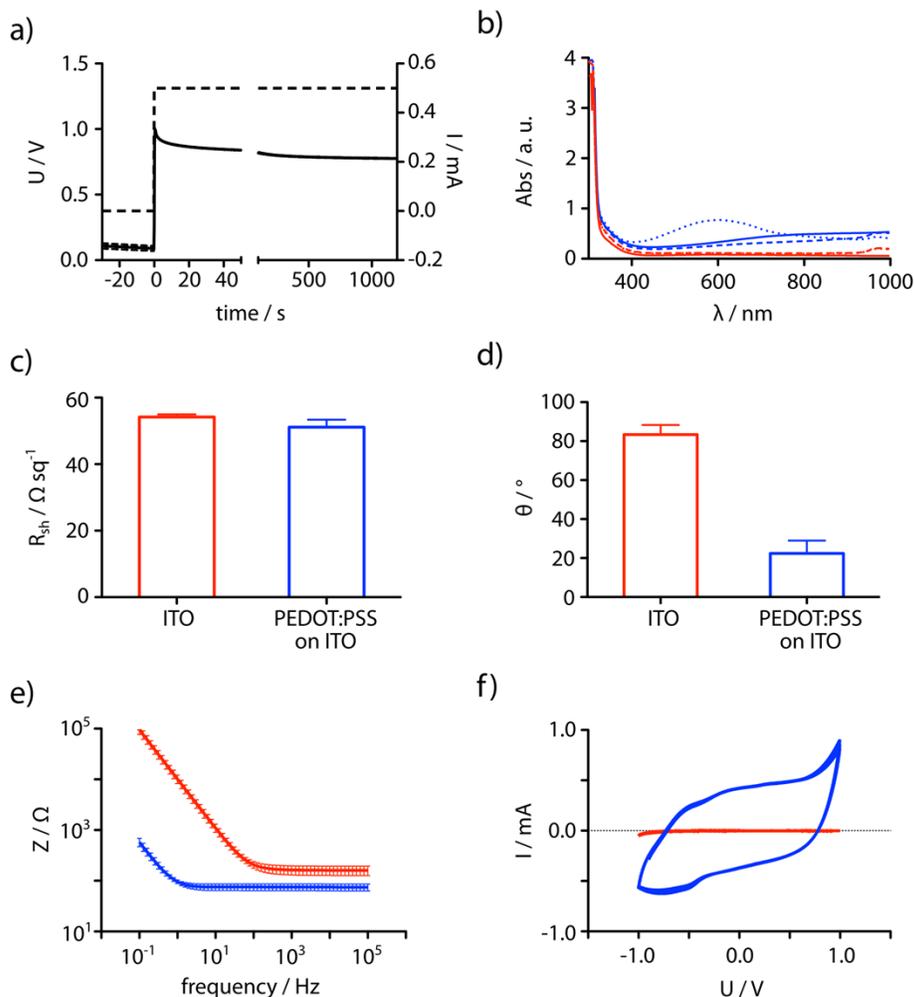


Figure S1. Characterization of deposited PEDOT:PSS layer versus ITO base

a) Chronopotentiometric recording (continuous line) during galvanostatic electrodeposition (dashed line) of EDOT with PSS counterions in aqueous solution (mean \pm sd, $n = 21$). **b)** UV-vis spectroscopy of ITO (red) and PEDOT (blue) surfaces in unbiased (continuous line), oxidated at +1.5 V potential (dashed line) and reduced at -1.5 V potential (dotted line) state (mean ($n=6$) without errors shown for clarity) **c)** Sheet resistance of ITO (red) and electrodeposited PEDOT:PSS (blue), (mean \pm sd, $n = 6$). **d)** Wettability as determined by contact angle measurement of ITO (red) and electrodeposited PEDOT:PSS (blue), (mean \pm sd, $n = 6$). **e)** Electrochemical impedance spectroscopy of ITO (red) and electrodeposited PEDOT:PSS (blue) at 0.1 Hz – 100 kHz in PBS electrolyte (mean \pm sd, $n = 4$). **f)** Cyclic voltammetry with 0.1 V/s to determine charge transfer capacity of ITO (red) and electrodeposited PEDOT:PSS (blue) in PBS electrolyte (4 cycles, shown are cycles 2 – 4, mean, $n = 6$).

Table S1.

Elements calculated from equivalent circuits fitted to impedance spectroscopy data.

Data for PEDOT:PSS in PBS and in fully supplemented DMEM are fitted with the equivalent circuit in **Figure 2d**. Data for PEDOT:PSS with a confluent layer of MDCK cells are fitted with equivalent circuit in **Figure 2e**.

	R_s	R_{ct}	C_{cpe}	α_{cpe}	C_{cells}	R_{cells}	χ^2
PBS	858.9±7.38	40.51±8.38	$3.2 \times 10^4 \pm 3.7 \times 10^{-6}$	0.96±0.006			9.84×10^{-5}
DMEM	773.5±3.98	81.22±53.1	$2.6 \times 10^4 \pm 6.6 \times 10^{-5}$	0.96±0.015			7.36×10^{-5}
MDCK	398.8±4.32	121.70±7.82	$9.7 \times 10^5 \pm 6.7 \times 10^{-6}$	0.64±0.014	$1.84 \times 10^{-4} \pm 7.87 \times 10^{-6}$	1766±67.53	3.20×10^{-4}

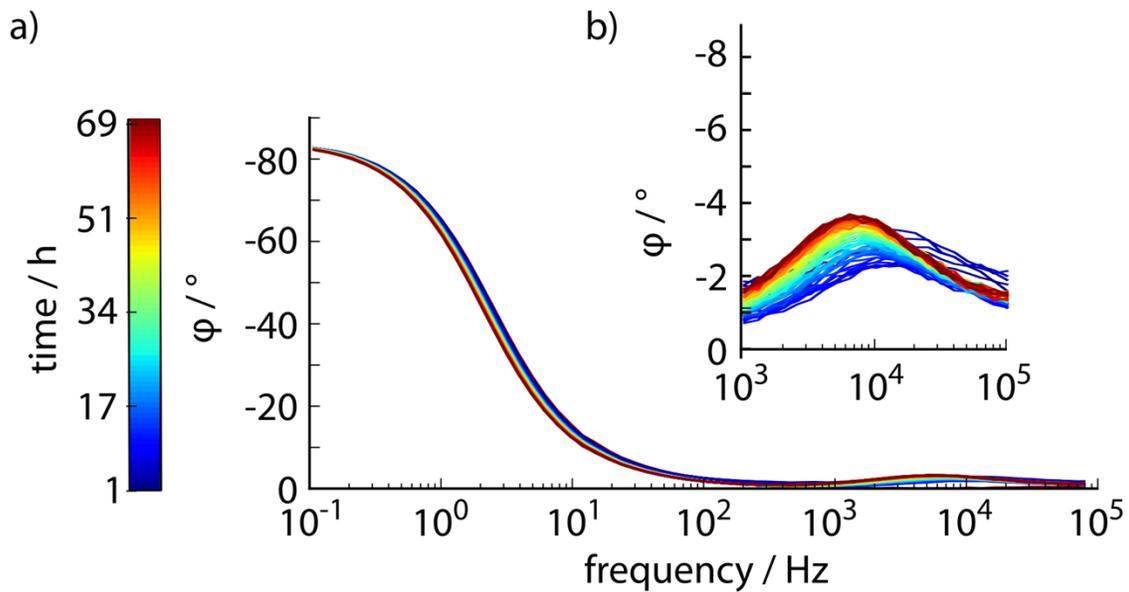


Figure S2. PAS spectra using fully supplemented DMEM as electrolyte.

(a) Control experiments using supplemented DMEM medium only (no cells). Full PAS spectra were recorded hourly for 70 h. Spectra are displayed as Bode plot with colour-coded time parameter. (b) Close-up of the frequency range 10^3 - 10^5 Hz.

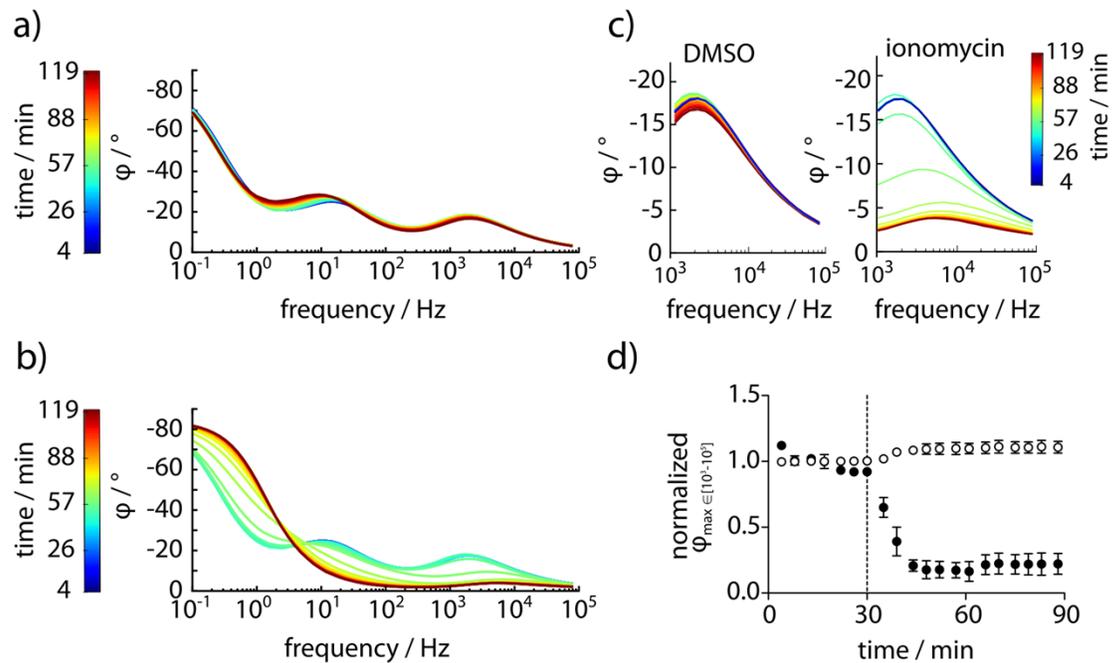


Figure S3. Epithelial monolayer disruption by full spectra PAS sensing.

(a) A confluent MDCK monolayer was treated with 20 μ l DMSO after 30 min baseline recording and full PAS spectra were recorded for 90 min in total. (b) A confluent MDCK monolayer was treated with 20 μ l ionomycin (1 mg ml⁻¹) in DMSO after 30 min baseline recording and full PAS spectra were recorded for 90 min in total. (c) Close-up of the ϕ peak amplitude in the 10³-10⁵ Hz frequency range for cells in DMSO, without (DMSO) and with (ionomycin) ionomycin treated cell layers. (d) Extracted and normalized ϕ peak amplitudes obtained from full PAS spectra for MDCK monolayers treated with ionomycin (full circles) and DMSO (open circles) after 30 min baseline recording indicating the confluency of the MDCK monolayer (n = 3).