

Real-Time Monitoring of Cellular Barrier Functionality with Dynamic-Mode Current-Driven Organic Electrochemical Transistor

Katharina Lieberth, Aristeia Pavlou, Daria Harig, Paul W. M. Blom, Paschalis Gkoupidenis, and Fabrizio Torricelli*

Cellular barriers control fundamental physiological functions in animals and plants. Accurate detection of barrier dysfunction requires real-time monitoring. Organic electrochemical transistors are a promising bioelectronic platform to monitoring cellular barriers. However, current approaches are not ideally suited for direct and real-time measurements: they require off-line model-based data analysis or slow measurement operation to achieve equilibrium conditions. Herein, dynamic-mode current-driven organic electrochemical transistors are proposed for direct real-time monitoring of cellular barrier functionality. In contrast to current approaches, the organic electrochemical transistor is operated under nonequilibrium conditions. The approach shows a sensitivity larger than $350 \times 10^{-6} \text{ V } (\Omega \text{ cm}^2)^{-1}$ with an operating range of $13\text{--}640 \Omega \text{ cm}^2$. The sensitivity can be optimized on-line by simply changing the dynamic conditions and real-time monitoring of reversible barrier functionality is demonstrated by using a tight-junction modulator with a concentration as-low-as $122 \times 10^{-6} \text{ M}$. The theoretical foundation of the method is provided. The analysis shows the general applicability of the approach, opening opportunities for precision in vitro bioelectronics and medical diagnostic.

of water and mineral nutrients,^[1,2] while in human body are the major building blocks of various organs, including, for example, skin, lungs, liver, kidney, and digestive track. The physiological function of the biological barriers is diverse among tissues and responds to the specific needs of each organ, including ion absorption, nutrients uptake, protection against toxins, and secretion of waste.^[3,4]

Under normal physiological conditions the transcellular and paracellular pathways are finely regulated by the cellular barriers. Tight-junction (TJ) proteins—being responsible of the intercellular sealing—control the paracellular fluxes, providing either fully impermeable barriers or permeable-selective functions.^[5–7] The permeability of TJs in a cell barrier can be regulated by physiological cues, can be modulated by drugs, and can be altered by various biological events such as inflammation, gastrointestinal tract diseases, cancer metastases, leukocyte migration,

and viral infections. For instance, disruption of epithelial and endothelial barriers is a key clinical data differentiating patients with high probability to develop severe COVID-19 symptoms including the escalation in respiratory deficiency, loss of viral containment, and a progression toward multiorgan dysfunction.^[8] Analogously, blood-brain barrier disruption contributes to the severity of diverse neurological diseases, including stroke, epilepsy, Alzheimer's disease, and multiple sclerosis, among others.^[9,10] More in general, monitoring cellular barriers with noninvasive and label-free methods is relevant for in vitro studies,^[11] for the development of organ-on-chip models,^[12] for studying a disease progression, and for drug testing and drug targeting, also promoting the replacement for animal testing in toxicological profiling.^[13]

So far, the integrity of cellular barriers has been addressed with transepithelial electrical resistance (TEER) measurements. TEER is an in vitro measurement technique based on two electrodes placed on each side of a cell layer. Upon the application of a direct or alternate current, the ionic impedance of the barrier layer is measured.^[14] TEER direct current method is easy to perform and the measurement provides a resistance value that depends on both the cell status and the electrode positions. The positioning of the electrodes is performed manually, and this

1. Introduction

Cellular barriers are essential biological interfaces for the physiological functions of animals, humans, and plants, being able to control the transport of ions, small molecules, and nutrients through the separated compartments of a tissue. For instance, cellular barriers in plants contribute to the controlled uptake

K. Lieberth, A. Pavlou, D. Harig, P. W. M. Blom, P. Gkoupidenis
Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany
F. Torricelli
Department of Information Engineering
University of Brescia
Via Branze 38, Brescia 25123, Italy
E-mail: fabrizio.torricelli@unibs.it

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/admt.202201697>.

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results in lab-to-lab (operator dependent) large variability and limited scalability. By recording the ionic impedance at various frequencies, electrochemical impedance spectroscopy (EIS) provides a more accurate approach.^[15,16] EIS is a well-established method for cell-layer status detection but unfortunately it requires time-consuming measurements and modeling, which limit the time resolution and increase the complexity. In addition, EIS measurements are based on small-amplitude signals, typically in the range of few millivolts, and a suitable signal-to-noise ratio demands additional filtering and amplification operations, which increase the complexity of the experimental set-up and the cost of the equipment.^[17]

In recent years, organic electrochemical transistors (OECTs) have been proposed as an effective alternative approach to electrically assess cellular barrier properties.^[18,19] OECTs are three-terminal iontronic devices where the electronic conductivity of an ionic–electronic conducting polymer, connected by two electrodes named source and drain, is modulated by a third electrode, named gate. The gate and the channel are in direct contact with an electrolyte and the polarity and magnitude of the applied gate voltage (V_G) give rise a drift of anions or cations from the electrolyte to the channel and vice versa.^[20–24] When an OECT is used to measure a barrier tissue, the biological barrier can be placed in-between the gate and the channel. In this configuration, the ion transport depends on the barrier status that, in turn, is mirrored by the electrical characteristics of the OECT. The pioneering work of Owens and co-workers,^[17] demonstrated the application of an OECT as a sensor for barrier tissue by applying a pulsed V_G and measuring the drain–source current I_D . This approach is suitable for long-term measurements but requires a model-based analysis of the recorded characteristics.^[25–27] To avoid the need of postprocessing, Hsing and co-workers proposed the direct coupling of cells' physiological ionic current.^[28] This approach was further optimized by Iannotta and co-workers that, focusing on the detection of irreversible barrier disruption, proposed the use of water as low ion concentration basal electrolyte but, unfortunately, a model-based analysis was re-introduced.^[29]

To monitor the reversible barrier functionality without the requirement of modeling and postprocessing analysis, we recently proposed the current-driven OECT configuration.^[30,31] This method can assess the integrity of cellular barriers when toxic compounds (e.g., H_2O_2) at a concentration of 10^{-3} M are added to the cell media. Very recently, using the same approach and optimizing the design in equilibrium conditions, we demonstrated the monitoring of reversible TJ modulations.^[32] Under equilibrium conditions the hysteresis in the electrical characteristics is minimized, avoiding a multivalued current when the same gate bias is applied. This is currently considered a stable measurement condition and provides a direct interpretation of the results.^[33–35] To minimize the hysteresis small device geometries and/or slow scan rate are required. Unfortunately, small device geometries are not an optimal design solution for the high-sensitivity monitoring of the cellular membranes^[31,36] while slow scan rates lead to large measurement time that, in turn, results in low time resolution. Therefore, while OECTs are a promising bioelectronic technology for the in vitro investigation of the cellular barrier functionalities, current approaches are not ideally suited for the real-time monitoring.

Here we propose real-time monitoring of cellular barrier functionality with dynamic-mode current-driven OECTs. In contrast to the current approaches, the OECT is deliberately operated under nonequilibrium hysteretic conditions. The dynamic-mode current-driven OECT enables cellular barrier monitoring with a sensitivity of $350 \times 10^{-6} \pm 10 \times 10^{-6}$ V ($\Omega \text{ cm}^2$)⁻¹ and an operating range of 13–640 $\Omega \text{ cm}^2$, extending from intact and high-resistivity to fully disrupted and low-resistivity biological barriers. The output sensitivity can be enhanced on-line and in real-time directly during the experiments, allowing the real-time monitoring of reversible TJs modulation using a modulator concentration as low as $c = 122 \times 10^{-6}$ M. The theoretical foundation of the proposed approach is explained by means of numerical simulations. Importantly, we highlight that the model-based simulations are essential to gain insight on the proposed method but are not needed for its practical application. The ultralow detection-limit combined with high-sensitivity and wide-range makes this approach a suitable technology for accurate monitoring subtle variations of barrier functionality.

2. Results and Discussion

2.1. Dynamic-Mode Current-Driven Organic Electrochemical Transistor

The current-driven OECT configuration integrating a barrier tissue is displayed in **Figure 1a**. In this configuration the OECT is connected in series with a current generator setting a bias current I_B . The output voltage V_O is measured at the drain and the input voltage V_I is applied to a Ag/AgCl quasi-reference electrode. A Transwell filter with a barrier tissue is placed in-between the quasi-reference electrode gating the OECT and the polymeric channel, thus separating the electrolyte into two compartments. The prototypical ionic–electronic conductive polymer poly(3,4-ethylenedioxy-thiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) is used for the OECT channel. PEDOT:PSS has been proven to be very stable in liquid environment even when in direct contact with the cell culture medium, thus ensuring the ideal biological conditions.^[37–39] The equivalent electronic circuit of the current-driven OECT embedding the Transwell filter with a biological membrane is displayed in **Figure 1b**. Specifically, R_A describes the nonpolarizable gate electrode and the ionic resistance due to the ion transport through the apical electrolyte, the barrier tissue membrane is modeled by a resistor R_M in parallel to a capacitor C_M , and R_F and R_B describes the ionic resistance of the Transwell filter and of the basal electrolyte, respectively. We note that R_M accounts for the ion transport through the cellular membrane while C_M models the ion accumulation at the apical and basal membrane interfaces.^[19] The aforementioned parameters are experimentally obtained by means of EIS measurements, as detailed in **Figure S1** of the Supporting Information. To probe the impedance of the cellular barrier, the experimental set-up should guarantee that $Z_{M-i} \gg (R_A + R_{\text{FILTER}} + R_B)$, where Z_{M-i} is the impedance of the intact cell barrier. The OECT channel is described considering the ionic–electronic volumetric capacitance and the charge transport in the electronic channel. The

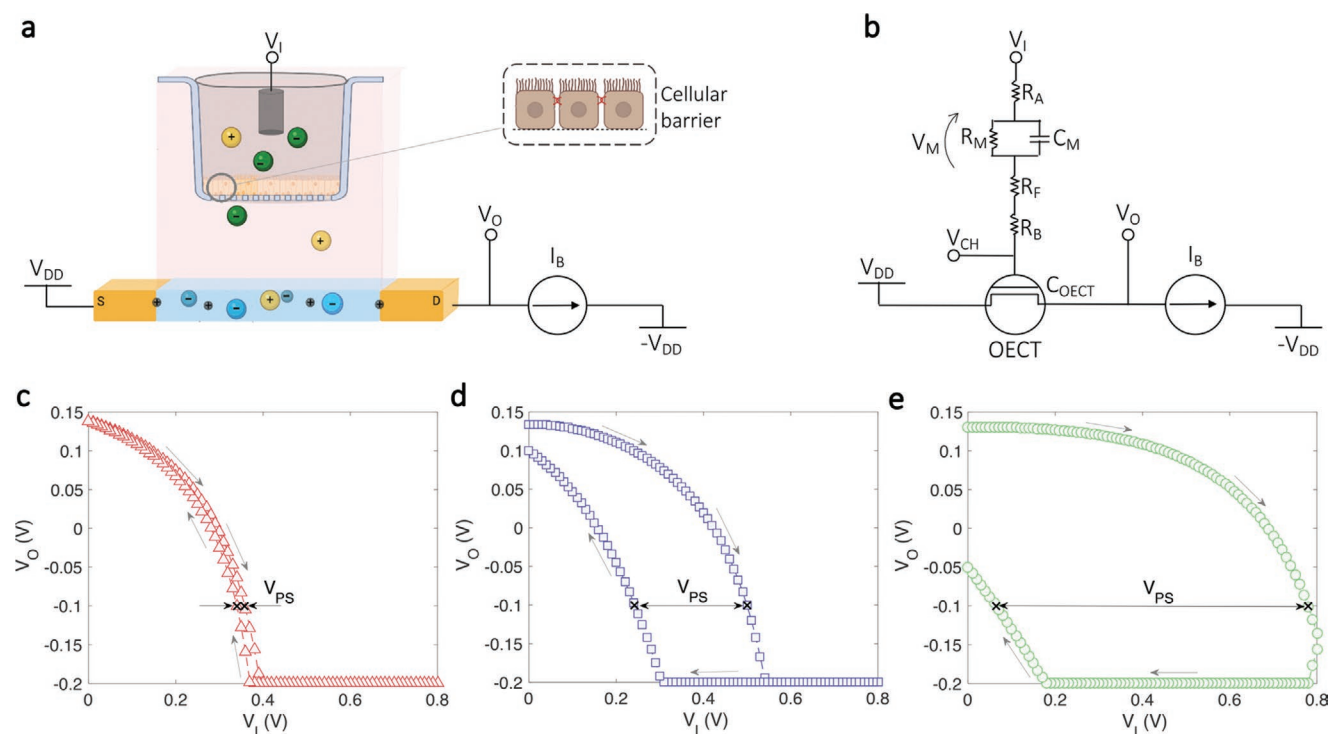


Figure 1. Dynamic-mode current-driven organic electrochemical transistor (OECT). a) Current-driven OECT configuration coupled with a biological barrier. The cellular barrier tissue is seeded in a Transwell filter and cell medium is used as electrolyte. The gate electrode is immersed in the apical compartment and the OECT channel is in contact with the basal compartment. The ion transport between the two compartments depends on the status of the barrier tissue. Created with BioRender.com. b) Equivalent circuit model of a dynamic-mode current-driven OECT coupled with a biological barrier tissue. The barrier tissue is described with a resistor R_M in parallel to a capacitor C_M . R_M accounts for the ion transport across the barrier and C_M models the ion accumulation at the apical and basal barrier interfaces. R_A and R_B are the ionic resistance of the apical and basal electrolyte, respectively. R_F is the ionic resistance of the Transwell filter and C_{OECT} is the overall capacitance of the OECT polymeric channel. V_I is the input voltage, V_{CH} is the voltage actually gating the OECT, V_{DD} is the supply voltage, I_B is the bias current, and V_O is the output voltage. The gray arrows show the forward and backward scans. c) Typical transfer characteristic V_O-V_I of a dynamic current-driven OECT operated at a scan rate $SR = 0.002 \text{ V s}^{-1}$. The phase-shift voltage V_{PS} is displayed. d) Measured V_O-V_I at $SR = 0.15 \text{ V s}^{-1}$. e) Measured V_O-V_I at $SR = 0.5 \text{ V s}^{-1}$. The gate is a Ag/AgCl pellet, the OECT geometries are: $W = 2 \text{ mm}$, $L = 1 \text{ mm}$, thickness $t = 300 \text{ nm}$. $I_B = 5 \times 10^{-3} \text{ A}$ and $V_{DD} = 0.2 \text{ V}$.

OECT parameters are extracted by modeling the transfer and output characteristics, as displayed in Figure S2 of the Supporting Information. In the following, the model depicted in Figure 1b will be used only to systematically understand the fundamentals of operation and model-based data analysis is not required for operation.

The idea is to monitor the status of the cellular barrier taking advantage of the transient response of the ionic–electronic biological system. A typical transfer characteristic (V_O-V_I) of the dynamic-mode current-driven OECT integrating a cellular barrier is displayed in Figure 1c. The input voltage V_I is swept forward and backward, from 0 to 0.8 V and back to 0.8 V, and the corresponding output voltage V_O is measured. When V_I is swept with a slow scan rate (i.e., by ensuring quasi-equilibrium conditions), e.g., $SR = 0.002 \text{ V s}^{-1}$ in Figure 1c, the forward and backward characteristics are almost perfectly overlapped. More in detail, when $V_I = 0 \text{ V}$ the p-type OECT is highly conductive and pulls-up the output voltage, resulting in $V_O = +V_{DD}$. By increasing the input voltage V_I , the OECT channel resistance (R_{OECT}) increases, I_B is set by the current generator, the voltage drop on the OECT channel ($V_{SD} = R_{OECT} I_B$) increases and, as a consequence, $V_O = V_{DD} - R_{OECT} I_B$ reduces. Further increasing V_I , the switching voltage V_{SW} is

achieved ($V_{SW} = 0.39 \text{ V}$ in Figure 1c), the OECT operates in saturation, and V_O is pulled-down to the minimum supply voltage ($V_O = -V_{DD}$). By increasing the scan rate of the input voltage, dynamic-mode conditions are obtained. Under these conditions the forward and backward characteristics do not overlap anymore and, as displayed in Figure 1d,e, the V_O-V_I characteristic shows a hysteric loop. The switching voltage of the forward characteristic (V_{SWf}) is larger than the switching voltage of the backward characteristic (V_{SWb}) and the voltage difference $V_{PS} = V_{SWf} - V_{SWb}$ increases by increasing SR. For instance, when $SR = 0.15 \text{ V s}^{-1}$ (Figure 1d). $V_{SWf} = 0.50 \text{ V}$, $V_{SWb} = 0.24 \text{ V}$, and $V_{PS} = 0.26 \text{ V}$, which increases to $V_{PS} = 0.72 \text{ V}$ when $SR = 0.5 \text{ V s}^{-1}$ (Figure 1e). It is worth to note that V_{PS} is calculated at $V_O = -0.1 \text{ V}$ because the output voltage $V_O = -0.13 \text{ V}$ at the maximum V_I and SR of the forward scan (Figure 1e). In general V_{PS} can be extracted at $V_O \geq V_{Omin(fw)}$, where $V_{Omin(fw)}$ is the minimum V_O of the forward scan.

This can be explained as follows. In the dynamic-mode current-driven OECT configuration, a triangular waveform with amplitude V_I and frequency f_I is applied as input. The frequency f_I is modulated by SR and reads: $f_I = SR \times (V_{max} - V_{min})^{-1}$, where V_{max} and V_{min} are the maximum and minimum amplitude of V_I , respectively. In our case $V_{min} = 0 \text{ V}$ and

$V_{\max} = 0.8 \text{ V}$ (Figure 1c–e). Considering the circuit model in Figure 1b, the amplitude and phase of the voltage actually gating the OECT channel, named V_{CH} , depends on the input frequency f_i and on the time-response of the R – C circuit accounting for the impedance of the cellular barrier (R_{M} , C_{M}), the electrolyte resistances (R_{A} and R_{B}), the Transwell filter resistance (R_{F}) and the OECT capacitance (C_{OECT}). A detailed calculation is provided in Note S1 of the Supporting Information.

2.2. Operation Mechanism

To gain insight on the operation of the dynamic-mode current-driven OECT configuration embedding a cellular barrier, we implemented the equivalent circuit displayed in Figure 1b in a numerical simulator accounting for the physical parameters obtained from the EIS, $I_{\text{D}}-V_{\text{G}}$, and $I_{\text{D}}-V_{\text{D}}$ measurements (Figures S1 and S2, Supporting Information). We note that a linear analysis cannot be applied because V_i is a large-signal triangular wave, and the current-driven OECT gives rise to a nonlinear input–output characteristic ($I_{\text{D}}-V_{\text{CH}}$). As a consequence, numerical nonlinear simulations are required. We highlight that the simulations are relevant to understand the bioelectronic system fundamentals but are not required when operating the system. As displayed in Figure 2a, the simulations accurately predict the measurements in the whole range

of V_i and SR. More in detail, we calculated the transient responses as a function of SR. For the sake of clarity, the various time responses are normalized to the corresponding SR. Figure 2b–e shows the input voltage (V_i), the potential drop across barrier tissue (V_{M}), the actual potential gating the OECT (V_{CH}), and the output voltage (V_{O}) as a function of the number of periods $N_{\text{p}} = \text{SR} \times \text{total time} \times ((V_{\text{max}} - V_{\text{min}}) \times 2)^{-1}$. When $\text{SR} = 0.002 \text{ V s}^{-1}$, V_{M} is negligible (Figure 2c, red line), V_{CH} shows the same amplitude and phase of V_i (Figure 2d, red line), and the forward and backward $V_{\text{O}}-V_i$ characteristics are almost overlapped (Figure 2a, red line). Therefore, for low SRs the cellular membrane is practically not probed. By increasing the scan rate at $\text{SR} = 0.15 \text{ V s}^{-1}$, the impedance of the OECT decreases, the voltage drop on the cellular membrane increases at about $V_{\text{M}} \approx 0.2 \text{ V}$ and the V_{M} oscillation has almost the same phase of the input signal V_i (Figure 2c, blue line). Conversely, V_{CH} is attenuated and shifted with respect to V_i (Figure 2d, blue line) and, as displayed in Figure 2e (green line), this is mirrored in a phase-shift of V_{O} . This behavior is confirmed by further increasing the scan rate at $\text{SR} = 0.5 \text{ V s}^{-1}$. In this condition, $V_{\text{M}} \approx 0.6 \text{ V}$ (Figure 2c, green line), V_{CH} is significantly attenuated and shifted with respect to V_i , and the corresponding V_{O} shows an increased phase-shift (Figure 2e, green line). As a result, the cellular membrane is probed for high SRs. Focusing on the amplitude of V_{O} , the maximum output voltage slightly decreases at the maximum SR while

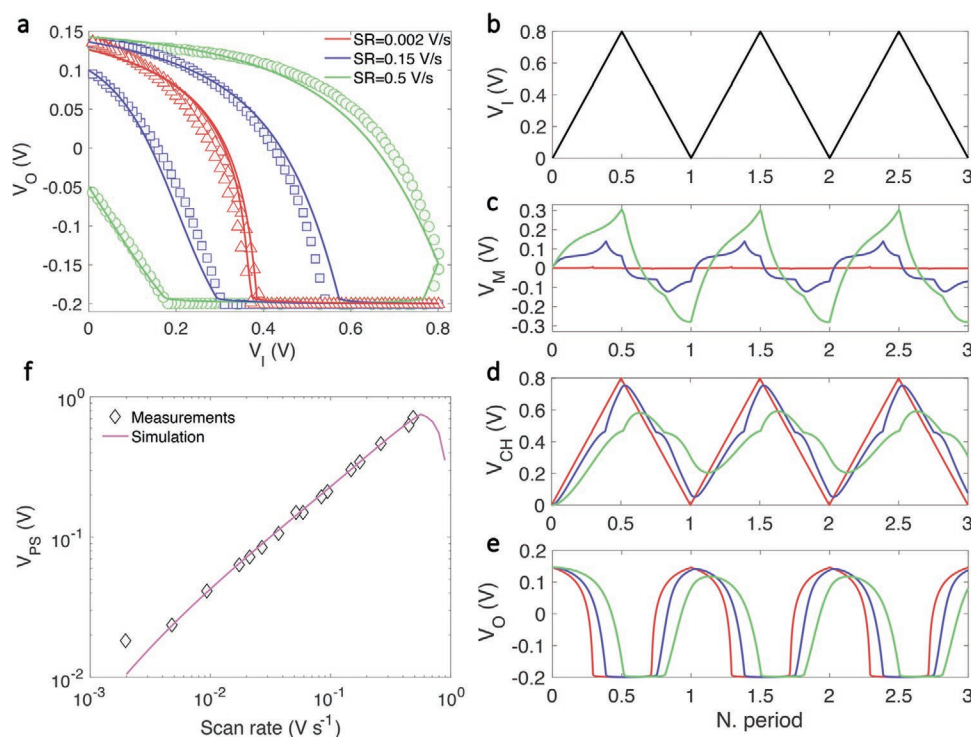


Figure 2. Operation mechanism. a) Measured (symbols) and model (lines) transfer characteristics ($V_{\text{O}}-V_i$) as a function of the scan rate $\text{SR} = 0.002 \text{ V s}^{-1}$ (triangles), $\text{SR} = 0.15 \text{ V s}^{-1}$ (squares), and $\text{SR} = 0.5 \text{ V s}^{-1}$ (circles). b–e) Simulations of dynamic-mode current-driven OECT with a cellular barrier at various SR: $\text{SR} = 0.002 \text{ V s}^{-1}$ (red line), $\text{SR} = 0.15 \text{ V s}^{-1}$ (blue line), and $\text{SR} = 0.5 \text{ V s}^{-1}$ (green line). Applied input voltage V_i , calculated voltage across the cellular barrier V_{M} , channel voltage V_{CH} , and output voltage V_{O} as a function of time normalized to the input frequency, number of period $N_{\text{p}} = \text{time} (V_{\text{max}} - V_{\text{min}}) \text{ SR}^{-1}$, where V_{max} and V_{min} are the maximum and minimum applied input voltage. Scan rate: $\text{SR} = 0.002 \text{ V s}^{-1}$ (red line), $\text{SR} = 0.15 \text{ V s}^{-1}$ (blue line), and $\text{SR} = 0.5 \text{ V s}^{-1}$ (green line). f) Phase-shift voltage V_{PS} as a function of the scan rate SR. Symbols are the measurements and line is calculated with the numerical simulations. Applied bias conditions: $I_{\text{B}} = 5 \times 10^{-3} \text{ A}$ and $V_{\text{DD}} = 0.2 \text{ V}$.

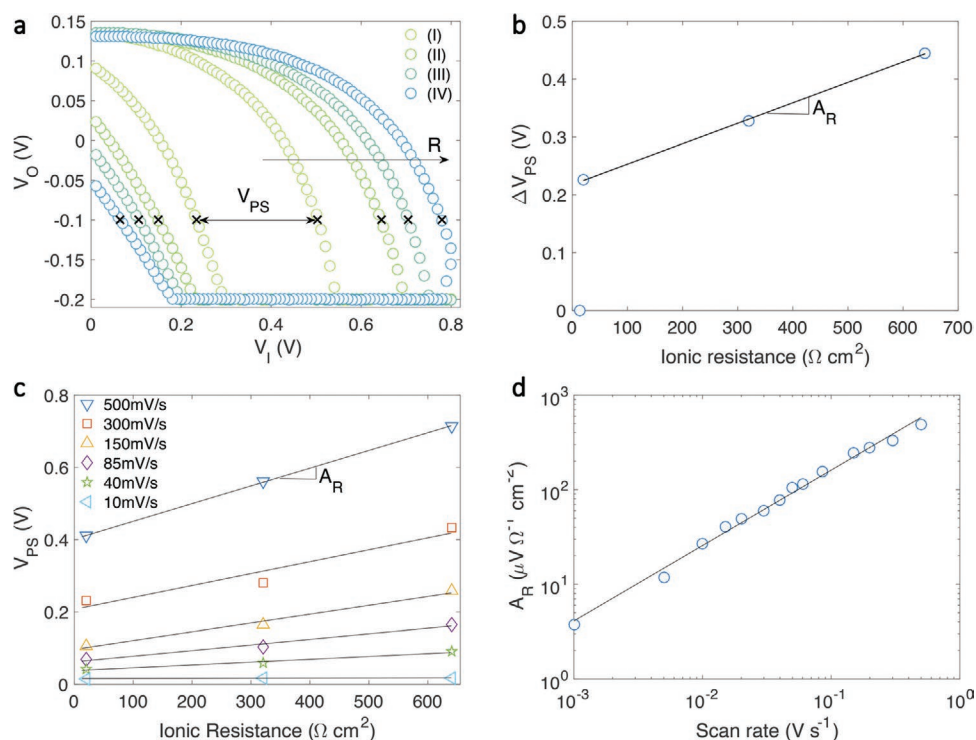


Figure 3. Dynamic reconfiguration of the performance. a) Transfer characteristics (V_O – V_I) measured as a function of ionic resistance R . Four relevant experimental cases are considered: (I) cell medium $R = 13 \Omega \text{ cm}^2$, (II) cell medium with Transwell filter $R = 20 \Omega \text{ cm}^2$, (III) low-resistance cellular barrier $R = 320 \Omega \text{ cm}^2$, and (IV) high-resistance cellular barrier $R = 640 \Omega \text{ cm}^2$. Considering the model displayed in Figure 1b, $R = R_A + R_M + R_F + R_B$. In all cases $SR = 0.5 \text{ V s}^{-1}$. The cross symbols indicate the forward and backward switching voltage for the various cases and, as an example, the phase-shift voltage V_{PS} is explicitly shown in the case (I). b) Variation of the phase-shift voltage $\Delta V_{PS} = V_{PS} - V_{PS(I)}$ as a function of the ionic resistance R . $V_{PS(I)}$ is V_{PS} obtained in the reference case (I). $SR = 0.5 \text{ V s}^{-1}$. Full line is the linear least square fit to the measurements and yields a sensitivity to the ionic resistance $A_R = (350 \pm 10) \times 10^{-6} \text{ V} (\Omega \text{ cm}^2)^{-1}$. c) Measured V_{PS} as a function of the ionic resistance R by varying SR showing that A_R consistently increases by increasing SR . d) Calculated average A_R as a function of SR . Applied bias conditions: $I_B = 5 \times 10^{-3} \text{ A}$ and $V_{DD} = 0.2 \text{ V}$.

the minimum output voltage is not altered ($V_{Omin} = -V_{DD}$, $V_{DD} = 0.2 \text{ V}$).

This behavior is inherently due to the OEET current-driven configuration: the pull-up toward V_{DD} depends on the overdrive voltage $V_{DD} - V_{CH}$ applied to the OEET, while the output pull-down toward $-V_{DD}$ is achieved by means of the current generator I_B . As a result, in a dynamic-mode current-driven OEET the voltage $V_{PS} = V_{SWF} - V_{SWb}$ is related to the input–output phase shift, providing a direct measurement of the ionic resistance of the cellular barrier. Indeed, as displayed in Figure S3 of the Supporting Information this hysteretic behavior disappears when the V_O – V_{CH} characteristics are plotted, confirming that V_{PS} provides information on the status of the barrier tissue. Interestingly, the provided analysis indicates that the sensitivity can be enhanced by means of SR . Figure 2f shows the measured V_{PS} (symbols) as a function of SR . V_{PS} systematically increases by increasing SR in the range 2×10^{-3} – $5 \times 10^{-1} \text{ V s}^{-1}$. At $SR = 0.5 \text{ V s}^{-1}$ we obtained $V_{PS} = 0.72 \text{ V}$, which is equal to 90% of the maximum input voltage. Overall, the dynamic-mode current-driven OEET shows a scan-rate sensitivity equal to $1391 \text{ mV V}^{-1} \text{ s}$. The simulations (Figure 2f, full line) accurately predict the measurements showing that the maximum performance is achieved. Indeed, further increasing SR the voltage drop on the cellular barrier increases as well, resulting in a decreasing of V_{CH} . As a consequence, although the phase-shift

increases, the OEET current reduces eventually resulting in a smaller V_{PS} (Figure S4, Supporting Information).

2.3. Operating Range

To experimentally investigate the operating range of the dynamic-mode current driven OEET, the ionic resistance between the gate and the channel is systematically varied considering four relevant cases: I) cell medium electrolyte only ($R = 13 \Omega \text{ cm}^2$), II) cell medium with Transwell filter ($R = 20 \Omega \text{ cm}^2$), III) low-resistance barrier ($R = 320 \Omega \text{ cm}^2$), and IV) high-resistance barrier ($R = 640 \Omega \text{ cm}^2$). We note that this range of resistances covers the relevant biological conditions, including intact and fully disrupted barrier tissues as well as the bare experimental set-up. Figure 3a shows the corresponding V_O – V_I measurements when $SR = 0.5 \text{ V s}^{-1}$. V_{PS} consistently increases by increasing the ionic resistance R . To quantitatively evaluate the effect of the ionic resistance on V_{PS} , we calculated $\Delta V_{PS} = V_{PS} - V_{PS(I)}$ where $V_{PS(I)}$ is the value of V_{PS} obtained in the reference case (I) where only the electrolyte is used. Figure 3b shows ΔV_{PS} as a function of ionic resistance R . V_{PS} increases by a factor of two in the experimental range we assessed. More in detail, by inserting the Transwell filter into the electrolyte, R increases from 13 to $20 \Omega \text{ cm}^2$ resulting in a $\Delta V_{PS} = 226.2 \text{ mV}$. ΔV_{PS}

increases to 327.8 mV when Transwell filter with a confluent barrier tissue of “low-resistance” Caco-2 cell line is measured and $R = 320 \Omega \text{ cm}^2$. $\Delta V_{\text{PS}} = 445.3 \text{ mV}$ when Transwell filter with “high-resistance” Caco-2 cells is measured and $R = 640 \Omega \text{ cm}^2$. The least-square linear approximation of the measured ΔV_{PS} as a function of R (Figure 3b) yields an average sensitivity $A_R = dV_{\text{PS}}/dR = 350 \times 10^{-6} \pm 10 \times 10^{-6} \text{ V } (\Omega \text{ cm}^2)^{-1}$. We note that case (I) is very relevant for evaluating the experimental set-up but it is of limited practical interest when monitoring the barrier tissues and therefore, to be conservative, it was not considered in the calculation of sensitivity. The dynamic-mode current-driven OECT shows an operating range of $13\text{--}640 \Omega \text{ cm}^2$, which is relevant to assess both fully disrupted and intact cellular barriers. Indeed, the characteristic TER range for confluent Caco-2 cell barrier is $140\text{--}700 \Omega \text{ cm}^2$.^[31,40–42] For instance, Tria et al. showed that the TER-value decreases to 20% of its initial value when Caco-2 cells are exposed to 10 mM EGTA,^[43] and Ramuz et al. demonstrated that TER-values below $100 \Omega \text{ cm}^2$ are typical of tissues without barrier function, whereas a TER from 100 to $300 \Omega \text{ cm}^2$ is obtained in the case of a partial barrier function.^[44]

To further assess the effectiveness of the proposed approach, we measured the $V_{\text{O}}\text{--}V_{\text{I}}$ input–output characteristics as a function of ionic resistance at various SR. Figure 3c shows that V_{PS} monotonically increases with R and the sensitivity A_R systematically increases by increasing SR. More in detail, the phase-shift between the input signal V_{I} and the voltage V_{CH} becomes larger at faster SR. The enhanced sensitivity to the ionic resistance R results in a larger V_{PS} . This is confirmed in Figure 3d where A_R as a function of SR is displayed. When the scan rate is very slow, $V_{\text{CH}} = V_{\text{I}}$, the signals show the same amplitude and phase, and it is not possible to detect the barrier status. By contrast, at high SR, e.g., $\text{SR} = 0.5 \text{ V s}^{-1}$, a large $V_{\text{I}}\text{--}V_{\text{CH}}$ phase shift is obtained because of the voltage partition between the ionic impedance of the barrier tissue and the ionic–electronic volumetric capacitance of the polymeric channel. This voltage partition is eventually converted in a large variation of V_{PS} by the dynamic-mode current-driven OECT. These results are further corroborated in Figure S5 of the Supporting Information, showing that the measured V_{PS} depends on R and the sensitivity can be significantly enhanced by means of SR.

2.4. Real-Time Monitoring of Cellular Barrier Functionality

As a relevant application, high-sensitivity real-time monitoring of cellular barrier functionality is demonstrated. As a model system, we used confluent barrier tissue of the Caco-2 cell line. Caco-2 cells are widely used as a model of intestinal barrier permeability, which is the major barrier separating our body from the external environment and it is essential to avoid any imbalance in homeostasis.^[45,46] For instance, an imbalance in the intestinal barrier can give rise to an uncontrollable immune reaction or various diseases including inflammatory disorders, rheumatoid arthritis, and metabolic disorders, e.g., obesity and diabetes.

As a first step, the dynamic-mode current-driven OECT approach is benchmarked with the state-of-art OECT approaches including transient response OECT measurements and conventional current-driven OECT.^[36,47] As displayed in

Figure 4a, the cell media is used as electrolyte and the electronic response is measured when a bare Transwell filter and a transwell filter with intact Caco-2 cell barrier are embedded in the current-driven OECT configuration. The OECT transient response and current-driven architectures are measured in the very same conditions, viz. same barrier and consequently. To directly compare the various architectures, we calculated the normalized output response $\text{NR} = 100 \times (\text{OR} - \text{OR}_0)/\text{OR}_0$, where in the case of transient response OR and OR_0 is the transient time with and without cells, respectively, in the case of conventional current-driven OR and OR_0 is the switching voltage V_{SW} measured with and without cells, respectively, and in the case of dynamic-mode current-driven OECT OR and OR_0 is the phase-shift voltage V_{PS} measured with and without cells, respectively. Figure 4b shows NR as a function of time for the various methods. The insertion of the cell barrier results in an $\text{NR} > 120\%$ in the case of dynamic-mode current-driven (DCD) OECT operated at $\text{SR} = 0.5 \text{ V s}^{-1}$. NR reduces to 40% when the conventional current-driven (CD) is used and lowers to $\text{NR} = 25\%$ in the case of the transient response method. This comparison shows that DCD provides an enhanced sensitivity with respect to the state-of-art OECT methods.

As a next step, cellular barrier functionality is monitored in real-time and in the very same biological conditions with the DCD and CD approaches. The relevant transfer characteristics $V_{\text{O}}\text{--}V_{\text{I}}$ measured with a DCD-OECT as a function of time are displayed in Figure 4c. The DCD-OECT with the intact barrier shows perfectly overlapped characteristics after 3 and 4 h of continuous measurements, proving the excellent stability of the bioelectronic system. Then, the barrier tissue is exposed to a low concentration of poly-L-lysine (PLL) TJ modulator, $c_{\text{PLL}} = 122 \times 10^{-6} \text{ M}$, and after $\approx 1.5 \text{ h}$ from the PLL modulator addition the TJs are opened (barrier disrupted). Ions can transport from the apical to the basal electrolyte compartment and vice-versa passing through the open channels of the cell barrier. The forward and backward characteristic shifts to smaller and larger V_{I} , respectively, and as a consequence the phase-shift voltage V_{PS} reduces. Then, the electrolyte cell media with PLL is exchanged (i.e., PLL is removed), the cellular barrier recovers, the $V_{\text{O}}\text{--}V_{\text{I}}$ characteristics shift back, and the corresponding V_{PS} increases. After 7 h upon medium replacement, cellular recovery is completed (Figure 4c, green line). A comparison with the initial characteristic of the intact barrier (blue line) suggests that a small amount of TJs could be still opened and a fully recovery after PLL exposure could not be achieved.

As a comparison, real-time monitoring of the barrier functionality with DCD and CD are displayed in Figure 4d. We note that DCD and CD require the very same experimental set-up, without performing manual operations during the whole time of the measurements. The output voltage V_{PS} and V_{SW} are normalized with respect to their value obtained when the barrier is intact ($t = t_0$, and $t_0 = 3 \text{ h}$ in Figure 4d), before exposure to PLL, and hence $\Delta V_{\text{PS}} = V_{\text{PS}}(t) - V_{\text{PS}}(t_0)$ and $\Delta V_{\text{SW}} = V_{\text{SW}}(t) - V_{\text{SW}}(t_0)$. The comparison of ΔV_{PS} and ΔV_{SW} as a function of barrier status demonstrates the real-time high sensitivity of the DCD. Indeed, CD shows a very limited response because of the very small concentration of PLL. By contrast, in the very same biochemical conditions the DCD architecture provides a response ΔV_{PS} of up to 216.2 mV and both the opening and

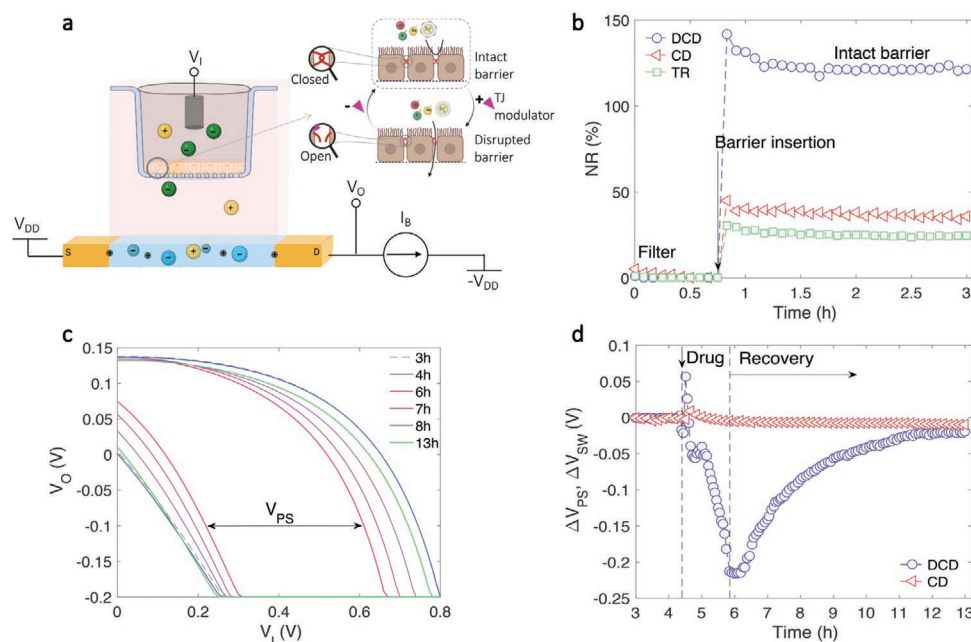


Figure 4. Real-time monitoring of cellular barrier functionality. a) Schematic representation of a current-driven OECT coupled with a biological barrier tissue, e.g., human Caco-2 cells. Ion transport through the barrier depends on the status of the tight junctions (TJs) proteins. Upon to the addition of a TJ modulator, inserted on a drug carrier, TJs open, the tissue becomes permeable to ions and drug carriers. By removing the TJ modulator in the electrolyte, the TJs are closed and the barrier functionality is restored limiting the ions and drug passage. Created with BioRender.com. b) Normalized response $NR = 100 \times (OR - OR_0) / OR_0$ as a function of time. In the case of transient response OR and OR_0 is the transient time with and without cells, respectively, in the case of conventional current-driven (CD) OR and OR_0 is the switching voltage V_{SW} measured with and without cells, respectively. The cell barrier is inserted into the electrolyte at time = 50 min and then the intact barrier is monitored for more than 2 h. c) Transfer characteristics ($V_O - V_I$) measured as a function of time. The intact barrier is measured two times at time $t = 3$ h and $t = 4$ h. Then the TJ modulator PLL, $c_{PLL} = 122 \times 10^{-6}$ M, is added to the cell medium. The measured barrier status is displayed at time $t = 6$ h where a large amount of TJs are open. Then the electrolyte cell media with PLL is exchanged, and the $V_O - V_I$ characteristics at $t = 7, 8, 13$ h show the recovery of the cellular barrier. d) Output signal measured with a DCD, $\Delta V_{PS} = V_{PS}(t) - V_{PS}(t_0)$, and CD, $\Delta V_{SW} = V_{SW}(t) - V_{SW}(t_0)$, as a function of time. The initial time is $t_0 = 3$ h. The CD shows a limited response because of the very small concentration of PLL. By contrast, the dynamic current-driven OECT provides a response ΔV_{PS} of up to 216.2 mV and both the disruption and recovery temporal dynamics are recorded in real-time and high sensitivity. The introduction of the filter with the barrier layer (panel b) and the PLL injection into the electrolyte (panel d) give rise to a fluid mixing that, for a limited period of time upon the perturbation, results in an increase of the ion mobility. The temporary increase of the ion mobility is mirrored by a spike of the V_{PS} .

recovery temporal dynamics can be recorded. To further assess the stability of the DCD experiments, we measured a Caco-2 cell barrier without any stress. More in detail, the DCD with a filter without the barrier is measured for ≈ 1 h. Then, the bare filter is replaced with a filter including a cell barrier. The DCD with cell barrier is measured for ≈ 12 h without any stress. The measured ΔV_{PS} as a function of time is shown in Figure S6 of the Supporting Information. The insertion of the filter with the barrier layer gives rise to a fluid mixing that results in an increase of the ion mobility and is mirrored by a peak of ΔV_{PS} . This is in full agreement with the results displayed in Figure 4d. After ≈ 20 min, the output signal ΔV_{PS} reaches a plateau and remains stable for the whole time of the experiment (≈ 11.5 h). We note that the maximum variation of the output signal amounts to $\Delta V_{PS}(t = 13 \text{ h}) - \Delta V_{PS}(t = 1.5 \text{ h}) = 0.01$ V, which is negligible compared to the signal variation measured during barrier disruption and recovery (> 0.2 V, Figure 4d). Finally, we note that the time period of our experiments (≈ 12 h) is suitable for acute toxicology or diagnostics purposes and, in agreement with previous studies,^[30,32,48,49] over this period of time we did not find a significant impact of the Ag/AgCl electrode.

3. Conclusion

Seeking for bioelectronic alternatives replacing the animal model on the in vitro platform, we proposed the dynamic-mode current-driven OECT configuration for high-sensitivity real-time monitoring of the cellular barrier functionality. This approach provides a direct real-time measurement of the cellular barrier status, with on-line reconfiguration and optimization of the bioelectronic system parameters.

The rational and operating principles of the proposed approach are investigated by means of numerical simulations, highlighting the key design parameters. Neither hardware post-processing analysis nor multiple-cycle design-optimization phases are required. The sensitivity can be dynamically reconfigured during the operation by simply changing the scan rate of the input signal, achieving optimal system performance in the whole range of biological conditions. A maximum sensitivity equal to $1391 \text{ mV V}^{-1} \text{ s}$ is demonstrated.

The experimental analysis of the DCD-OECT provided an average ionic resistance sensitivity equal to $350 \times 10^{-6} \pm 10 \times 10^{-6} \text{ V } (\Omega \text{ cm}^2)^{-1}$ with an operating range of $13\text{--}640 \text{ } \Omega \text{ cm}^2$.

The high sensitivity combined with the direct output response and easy reconfiguration even during the real-time operation, allowed us to accurately monitor barrier disruption and recovery in human Caco-2 cells using PLL modulator at a concentration down to 122×10^{-6} M. The DCD approach is benchmarked under the very same biochemical conditions with the CD approach, highlighting the superior performance of the DCD-OECT method.

Prospectively, the proposed approach could be used as-it-is for real-time multiparametric in vitro cell monitoring including cell layer coverage, cellular vitality, differentiation, ionic channel molecular transport, and cell toxicity experiments. In addition, the proposed technological implementation can be extended to other electrochemical transistor material technologies including, for example, n-type and ambipolar organic mixed ionic–electronic conductors, as well as accumulation and depletion mode OECTs.^[35,50–53] Overall, the high sensitivity combined with the reconfigurable operation and simple fabrication on flexible or even conformable substrates can open opportunities for next-generation organic bioelectronics.

4. Experimental Section

Device Fabrication: Glass substrates were cleaned by sonication in DI water with soap (Micro-90) and in a 1:1 (vol/vol) solvent mixture (acetone, isopropanol), followed by drying and UV ozone cleaning. Gold source and drain electrodes with chromium as an adhesion layer (5 nm) were deposited by thermal evaporation using a shadow mask for defined channel dimensions ($W = 2$ mm, $L = 1$ mm). The PEDOT:PSS formulation consisted of an aqueous dispersion of the conducting p-type polymer PEDOT:PSS (Clevios PH1000), mixed with 5 vol% ethylene glycol, 0.1 vol % dodecyl benzene sulfonic acid, and 1 vol% (3-glycidyloxypropyl) trimethoxysilane], and spin-coated first at 1500 rpm and then two times at 650 rpm for 60 s with an annealing step of 120 °C for 1 min in between the first two steps. The devices were baked 1 h at 140 °C, then glass slides were soaked in DI water for 1 h. To contain the electrolyte, a polymethyl methacrylate-well was placed on top of the device using double-sided tape.^[54,55]

Device Measurements: All electrical measurements were performed in a humidified atmosphere with 5% CO₂ by using a Keithley 4200-Semiconductor Characterization System and were analyzed by using OriginLab software. Cell culture medium (Eagle's minimum essential medium, EMEM) was utilized as an electrolyte apical and basal. A Ag/AgCl electrode (pellet, 2 mm, Warner Instruments) operated as a gate, was immersed in the Transwell filter. The operating gate voltage was kept well below 1.0 V to avoid water electrolysis and any cell damage. For the dynamic current-driven configuration a dual sweep of the input voltage $V_{IN} = [0; 0.8]$ V was performed at the gate. The scan speed was chosen in the range of $[0.002; 0.5]$ V s⁻¹. For the standard current-driven measurement the input voltage was swept forward only and the scan rate $SR = 0.125$ V s⁻¹. The supply voltage was kept same for both techniques at $V_{DD} = 0.2$ V. For transient response measurements V_C was pulsed at 0.5 V ($t_{OFF} = 3$ min, $t_{ON} = 15$ s) and V_D was kept constant at $V_D = -0.2$ V. These parameters are in agreement with previous studies.^[26,30,56] The transient response time was extracted by an exponential fit of $I_D(t)$ using the following equation: $I_D = A_1 \exp(-t/\tau_1) + I_{D0}$, where τ_1 is the dominant time constant. A single exponential model where the time constant τ_1 accounts for the device response time influenced by the cell layer status was used.^[26,30,36,48,56] Comparing dynamic current-driven, current-driven, and transient response measurements, a waiting time of 3 min was imposed between each sequence of measurement.

Cell Culture: Caco-2 cells (DSMZ, ACC 169) were seeded at 1.5×10^5 cells per insert in a Transwell filter (1.12 cm², pore size = 0.4 μm). The filter area was reduced to ≈0.16 cm² using PDMS coating on the

backside to lower its ion permeability. The PDMS-modified Transwell filters were coated with collagen, as stated in the literature for enhanced cell attachment, prior to cell seeding. Cells were cultured in EMEM (Invitrogen) with 10% fetal bovine serum (FBS, Invitrogen), 2 mM glutamine (GlutaMax-1, 100×, Invitrogen) and Pen-strep (10 000 μg mL⁻¹ penicillin, 10 000 μg mL⁻¹ streptomycin, Invitrogen) with a medium change every 2 or 3 days. During the experiments, as well as cell culturing, cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂. Four days after seeding cells on Transwell filters of polycarbonate membranes a confluent cell layer is obtained^[30,40,41] and the measured TER-value is due to the filter ($TER = 10 \Omega \text{ cm}^2$). Five days after seeding, the TER increases with the presence of TJs to ≈100 $\Omega \text{ cm}^2$ and TER values in the range of 140–700 $\Omega \text{ cm}^2$ are achieved.^[31,40–44] As explained by Volpe,^[40] the TER depends on various cell culture conditions, such as culturing days, buffer compounds, passage number, etc. In this work, samples with different TER-values were obtained from the same batch. To investigate the DCD approach, “low-resistance” cellular barrier $R = 320 \Omega \text{ cm}^2$, and “high-resistance” cellular barrier $R = 640 \Omega \text{ cm}^2$ were selected. To determine the TER-value, barrier tissue samples were measured using a Volt–Ohm meter EVOM2 from World Precision Instruments.

Caco-2 Cell Exposure to PLL in Transwell Filter: Experiments were performed after day 14, corresponding to a TER of ≈500 $\Omega \text{ cm}^2$. The TER was measured with a Volt–Ohm meter EVOM2 from World Precision Instruments. The cells during the experiments were not monitored with the TER because this would require changing the experimental set-up. The cellular barrier with DCD and CD methods was monitored by using the very same experimental set-up without performing manual operations during the measurements. During the monitoring of the cellular barrier, 90 μL PLL dissolved in DI water (122 μM, Sigma-Aldrich) was injected in the apical cell medium and it was verified that the variation of the total ion concentration was negligible. During the whole experiment, the samples were kept in a humidified atmosphere with 5% CO₂.

Data Presentation: The data are presented as one set of experiment. Additional experiments were designed for reproducibility. Among all measurements, the overall behavior (electrical characterization) was reproducible; Software: OriginLab was used for the statistical analysis, and Matlab was used for the data analysis and presentation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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biosensors, organic bioelectronics, organic electrochemical transistors, real-time cellular membrane monitoring

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Supporting Information

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Real-Time Monitoring of Cellular Barrier Functionality
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*Katharina Lieberth, Aristeia Pavlou, Daria Harig, Paul W.
M. Blom, Paschalis Gkoupidenis, and Fabrizio Torricelli**

Real-Time Monitoring of Cellular Barrier Functionality with Dynamic-Mode Current-Driven Organic Electrochemical Transistor

Katharina Lieberth^a, Aristeia Pavlou^a, Daria Harig^a, Paul W.M. Blom^a, Paschalis Gkoupidenis^a, Fabrizio Torricelli^{b*}

^a Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

^b Department of Information Engineering, University of Brescia, Via Branze 38, 25123 Brescia, Italy

e-mail: fabrizio.torricelli@unibs.it

Pag. 2 Figure S1: Electrochemical impedance spectroscopies.

Pag. 3 Figure S2: Electrical characteristics of OECTs without and with cellular barrier tissue.

Pag. 4 Figure S3: Impact of the scan rate on the OECT characteristics.

Pag. 5 Figure S4: Impact of the scan rate on the V_O - V_I characteristics.

Pag. 6 Figure S5: Impact of the scan rate and ionic resistance.

Pag. 7 Figure S6: Output signal ΔV_{PS} without and with cellular barrier.

Pag. 8 Note S1: Model of the dynamic-mode current driven OECT used for the numerical circuit simulations

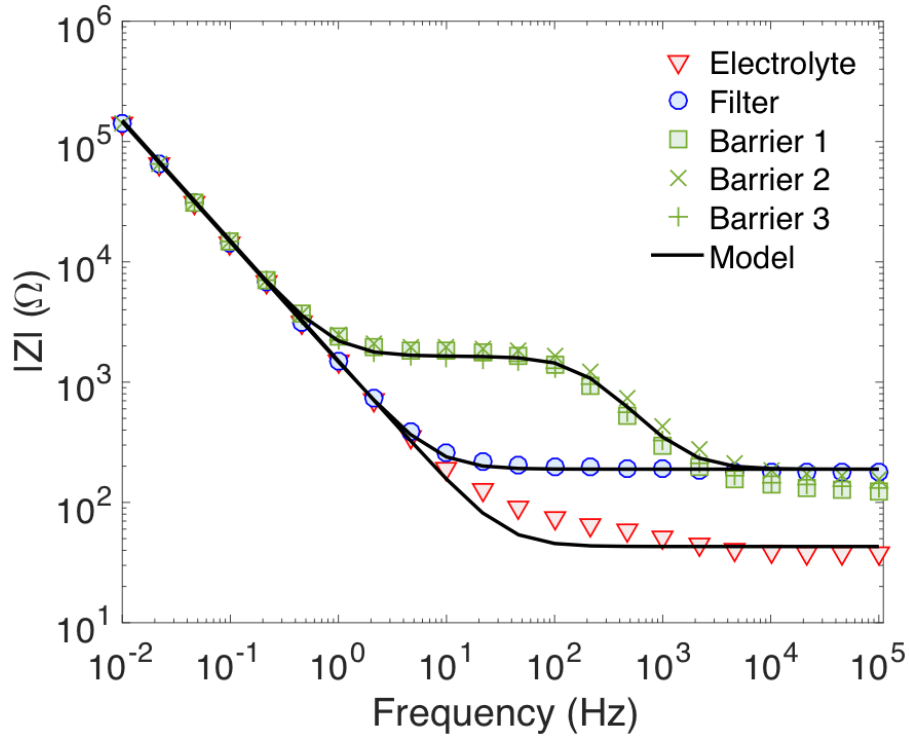


Figure S1. Electrochemical impedance spectroscopies. **a** Typical electrochemical impedance spectroscopy (EIS) of OECTs with cell medium used as electrolyte (triangles), OECT with transwell filter (circles), OECT with transwell filter and various nominally identical barrier tissue tissues (square, cross, plus). The full line is calculated with the lumped circuit model in Fig. 1b, and the bio-system parameters are extracted. The various EIS enabled us to disentangle the various parameters. More in detail, the EIS of the bare OECT with the cell culture medium used as electrolyte provided R_A , R_B and C_{OECT} , the EIS of the OECT with the transwell filter provided R_F and the EIS of the OECT with the various cell barrier tissues yields R_M and C_M . In our experiment $R_A + R_B = 28 \, \Omega$, $R_{FILTER} = 145 \, \Omega$, $C_{OECT} = 109 \, \mu F$, $R_M = 1750 \, \Omega$ (intact barrier), and $C_M = 0.47 \, \mu F$ (intact barrier).

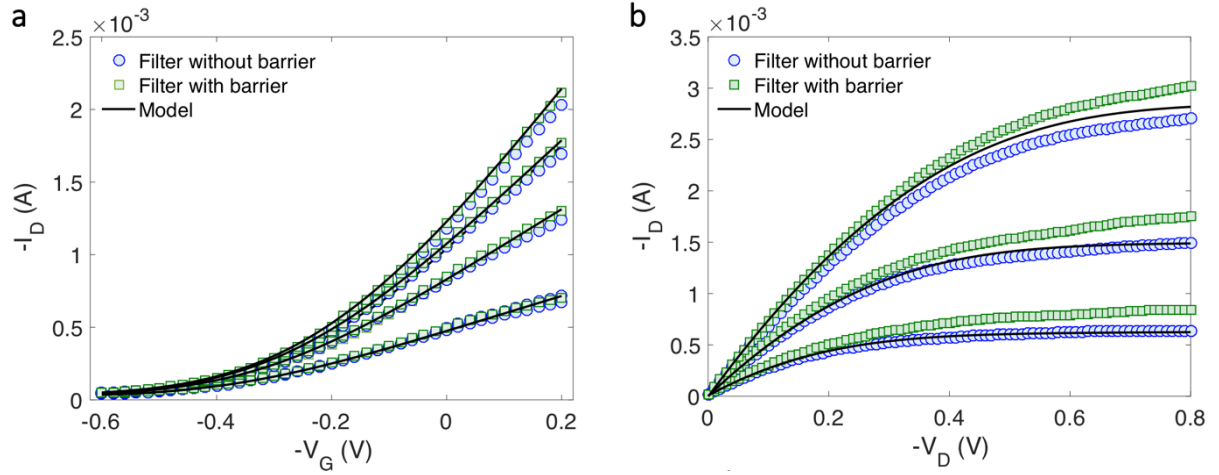


Figure S2. Electrical characteristics of OECTs without and with cellular barrier tissue. **a** Typical transfer characteristics and **b** typical output characteristics of OECTs measured without (circles, $\text{TER} = 13 \, \Omega \, \text{cm}^2$) and with cellular barrier tissue (squares, $\text{TER} = 640 \, \Omega \, \text{cm}^2$). Symbols are the measurements and lines are calculated with the OECT model proposed in ref. [1] including the channel length modulation.^[2,3] In all cases the cell media is used as electrolyte. The OECT characteristics without and with the cellular barrier tissue are almost perfectly overlapped showing that the barrier tissue has a minor impact on the steady-state electrical characteristics. The measurements are accurately described by the OECT model providing the channel parameters. We emphasize that the model depicted in Fig. 2b will be used only to systematically understand the fundamentals of operation. The proposed approach does not require model-based data analysis and provides direct real-time output.

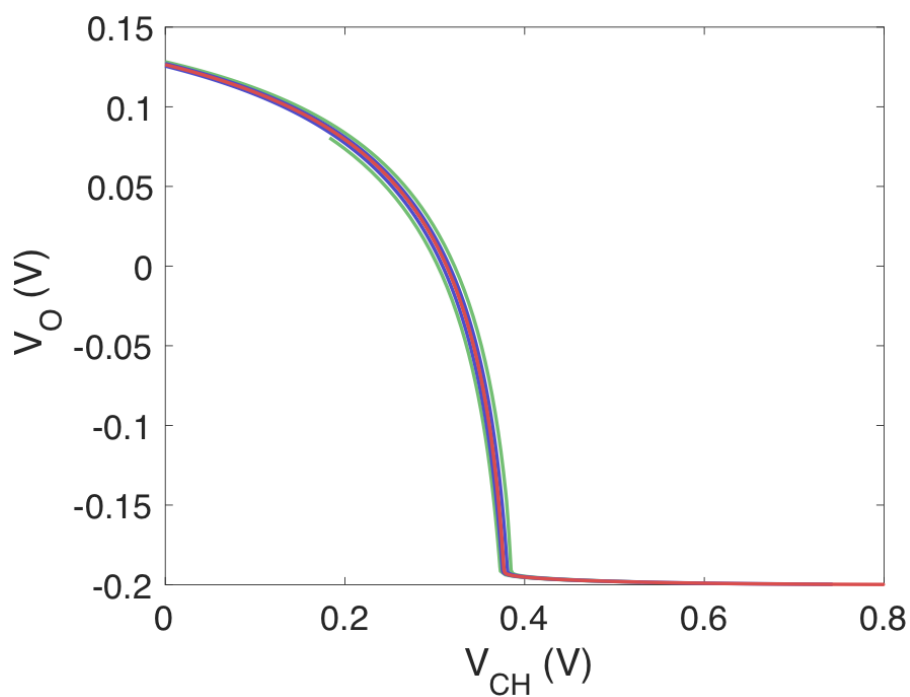


Figure S3. Impact of the scan rate on the OECT characteristics. Output voltage V_O as a function of the channel voltage V_{CH} at various scan rate SR. SR = 0.002 V s^{-1} (red line), SR = 0.15 V s^{-1} (blue line), and SR = 0.5 V s^{-1} (green line). The various characteristics are almost perfectly overlapped showing that the OECT response is independent of SR and the phase-shift is inherently due to the cellular barrier tissue.

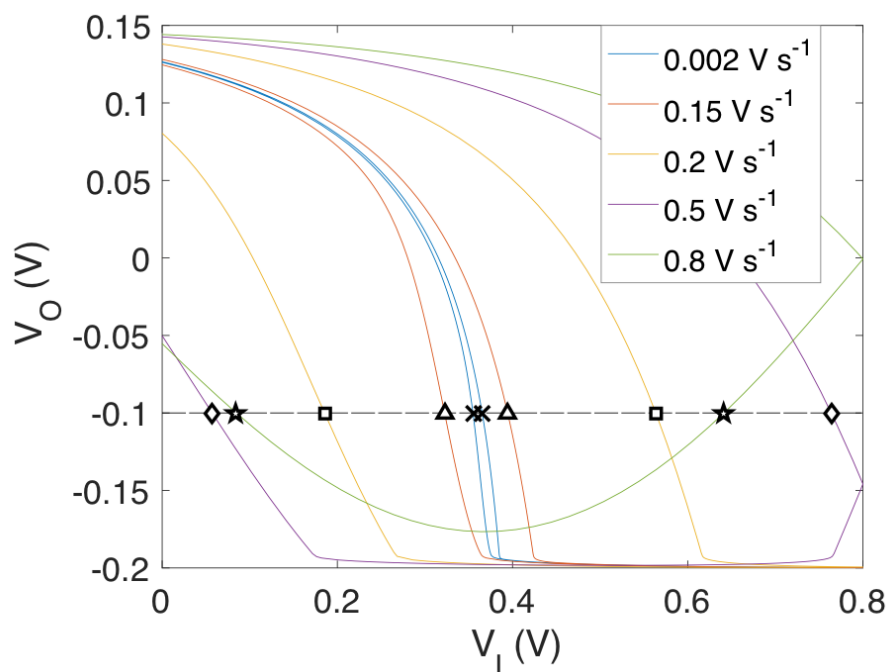


Figure S4. Impact of the scan rate on the V_O - V_I characteristics. V_O - V_I transfer characteristics as a function of the scan rate SR. The parameters of the dynamic-mode current-driven OECT are the same of Fig. 2. The symbols highlight V_{PS} for each curve obtained with a different SR. By increasing SR from 0.002 V s^{-1} to 0.5 V s^{-1} , V_{PS} consistently increases (crosses, triangles, squares, and diamonds) while at larger SR, e.g. $SR = 0.8 \text{ V s}^{-1}$ V_{PS} decreases (stars).

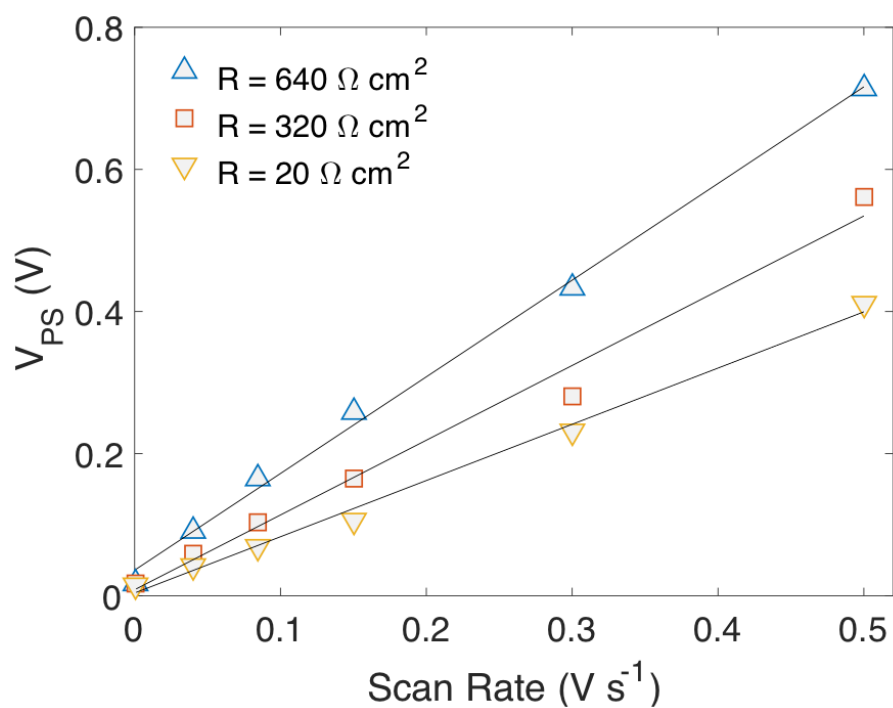


Figure S5. Impact of the scan rate and ionic resistance. Phase-shift voltage V_{PS} measured as a function of the scan rate SR by varying the experimental ionic resistance R . V_{PS} consistently increases by increasing SR and R .

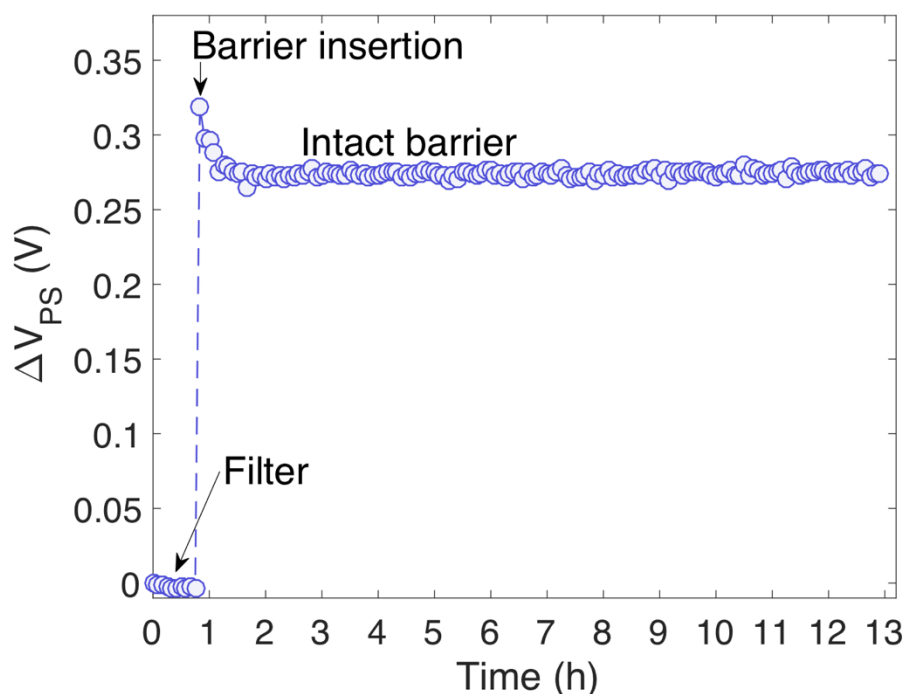


Figure S6. Output signal ΔV_{PS} without and with cellular barrier. The output signal ΔV_{PS} is measured as a function of time. DCD is measured with a bare filter (without cellular barrier) for about 1 hour. Then, the bare filter is replaced with a filter with cell barrier. The DCD with cell barrier is measured for about 12 hours without any stress. The sampling time was 5 minutes. The insertion of the filter with the barrier layer gives rise to a fluid mixing that results in an increase of the ion mobility and is mirrored by a peak of ΔV_{PS} . After about 20 minutes upon the insertion of the cell barrier, the output signal ΔV_{PS} reaches a plateau and remains stable for the whole time of the experiment (about 11.5 hours). The maximum variation of the output signal amounts to $\Delta V_{PS}(t=13h) - \Delta V_{PS}(t=1.5h) = 0.01V$.

Note S1. Model of the dynamic-mode current driven OECT used for the numerical circuit simulations

According to the circuit model displayed in Fig. 1b, the channel voltage V_{CH} can be related to the input voltage V_I and output voltage V_O as follows:

$$V_{CH} = H_1(s)V_I + H_2(s)V_O \quad (1)$$

where

$$H_1(s) = \frac{1}{1+s Z_I C_{OECT}} \quad (2)$$

$$H_2(s) = \frac{s Z_I C_{OECT}}{2} H_1(s) \quad (3)$$

$$Z_I(s) = R_A + \frac{R_M}{1+s R_M C_M} + R_F + R_B \quad (4)$$

$s = j 2 \pi f$, $j = \sqrt{-1}$ and f is the frequency.

Then, the electronic current flowing through the polymeric channel (I_D) is calculated using the Bernards-Malliaras model^[1] including the channel length modulation,^[2,3] and considering the current-driven circuit topology ($I_D = I_B$) it reads:

$$I_B = \Gamma \left(V_{DD} - V_{CH} + V_T - \frac{V_{DD} - V_O}{2} \right) (V_{DD} - V_O) \quad \text{if} \quad V_{CH} - V_T < V_O \quad (5)$$

$$I_B = \Gamma (V_{DD} - V_{CH} + V_T)^2 [1 + \lambda (V_{DD} - V_O)] \quad \text{if} \quad V_{CH} - V_T \geq V_O \quad (6)$$

where $\Gamma = \left(\frac{W}{L} \right) t_p \mu C_V$, W and L is the channel width and length, respectively, t_p is the thickness of the polymeric channel, μ is the electronic mobility and C_V the volumetric capacitance. I_B is the bias current set by the current generator, V_{DD} is the supply voltage, V_T is the OECT threshold voltage and λ accounts for the channel length modulation.

The set of non-linear equations (1), (5) and (6) are coupled and solved together as a function of time. The transient simulations eventually provide the V_O - V_I transfer characteristics of the dynamic-mode current-driven OECT. According to the measurements, in our simulations we applied as input voltage V_I

a large-signal triangular waveform with frequency $f_i = SR (V_{\max} - V_{\min})$, where SR is the scan rate, and $V_{\max}$ and $V_{\min}$ are the maximum and minimum amplitude of V_i , respectively.

References

- [1] Bernards, D. A. & Malliaras, G. G. Steady-state and transient behavior of organic electrochemical transistors. *Adv. Funct. Mater.* 17, 3538–3544 (2007).
- [2] Romele, P., Ghittorelli, M., Kovács-Vajna, Z. M. & Torricelli, F. Ion buffering and interface charge enable high performance electronics with organic electrochemical transistors. *Nat. Commun.* 10, 3044 (2019).
- [3] Torricelli, F. et al. Transport Physics and Device Modeling of Zinc Oxide Thin-Film Transistors - Part II: Contact Resistance in Short Channel Devices. *IEEE Trans. Electron. Dev.* 58, 3025-3033 (2011).