An Organic Electrochemical Transistor to Monitor Salmonella Growth in Real-Time

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Organic electrochemical transistors (OECTs) are used in research and diagnostic applications due to their facile manufacture, scalability, and biocompatibility. In these devices, the source–drain current upon gate voltage application depends on ion concentration in the electrolyte. This study investigates whether an OECT can be employed to monitor bacterial growth since it is known that the concentration of charged species increases in bacterial cultures during growth. A poly(3,4-ethylenedioxythiophene):polystyrene sulfonate-based single-well OECT, compatible with long-term incubation of bacterial cultures, is fabricated. It is shown that the growth of *Salmonella* alters the transfer characteristics of the device and demonstrates how it can be applied to monitor growth in real-time by recording the source–drain current at gate voltage +0.5 V. The signal can also be measured in filtrates of bacterial cultures, devoid of bacterial cells. This suggests that the signal originates from charged metabolic products. Bacterial biofilm formation does not alter the device response. This proof-of-principle study presents OECT recordings as an alternative to optical methods, allowing bacterial growth to be monitored in transparent and opaque media alike. By measuring metabolic products rather than bacterial cell multiplication, insight into the stationary phase and other nondividing states may be obtained in the future.

1. Introduction

Electrochemical methods are of importance for microbial diagnostics and are commonly employed in biosensors.[1–3] Among the most known and widely used applications is impedance, which took off as a diagnostic method in the 1970s, following the introduction of dedicated impedance systems.[4] In its simplest form, the AC impedance at a single frequency was measured at a pair of electrodes immersed in a bacterial culture.[5] As bacteria grow, the conductivity of the culture medium changes[6] as a consequence of bacterial metabolism, whereby uncharged substrates are transformed into charged metabolites.[4,7] This in turn leads to changes in impedance.[5] Impedance was shown to be superior to commonly used colony forming units counting for bacterial detection in urine[8] and blood.[9,10] Conductance of the medium was found to be well correlated with the growth of bacteria monitored by absorbance.[11] Despite the advances in the field, only a few impedimetric sensors were commercialized, mainly due to unsatisfactory detection limits and high production costs.[5] The discovery of conjugated polymers in 1977, and the emergence of organic bioelectronics, have provided the scientific community with low-cost and easily processable materials that are capable of ionic and electronic transport.[12,13] This has led to the development of innovative approaches and novel devices for microbiology and infection research.[14–17]

Organic electrochemical transistors (OECTs), first described in 1984,[18] consist of three electrodes—source, drain, and gate. An organic semiconductor film between the source and drain is in contact with an electrolyte that connects the film to the gate electrode.[19] The most common semiconductor in OECT research is poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS).[20] Application of a positive gate voltage ($V_G$) causes migration of cations into the PEDOT:PSS film, changing its conductivity and, therefore, the current flowing between the source and drain electrodes ($I_{SD}$).[19] PEDOT:PSS OECTs are commonly seen as signal amplifiers due to their high ON/OFF ratios, i.e., relatively small variations of $V_G$ can lead to substantial changes in $I_{SD}$.[19–21] Most applications to date employ them to i) amplify the signals from electroactive cells (e.g., neurons,[22] cardiomyocytes,[23]) ii) monitor barrier tissue integrity[24,25] or monolayer formation,[19,26] and iii) detect analytes.[19,27,28] The gate current is typically altered by affecting $V_G$[22,23,27] or by presenting a physical barrier between the gate electrode and the channel.[24,25,28] While the effect of ion concentration on $I_{SD}$ is well known,[19,26] this phenomenon is rarely applied in the OECT field. Moreover, there are only a few reports of OECTs being used in microbiology research and diagnostics.[31–33]

Inspired by impedance microbiology, we investigated whether OECT can be used to monitor bacterial growth by...
detecting an increase in ionic strength of the medium. We fabricated PEDOT:PSS-based single-well OECTs (Figure 1) in which we grew Salmonella Enteritidis and monitored the OECT response over time. To control for possible effects of extracellular matrix (ECM) associated with biofilm formation, the wild type (WT) bacterium was compared to an isogenic mutant lacking csgD, the gene coding for a major regulator of biofilm formation. Finally, we employed scanning electron microscopy (SEM) to study the morphology of bacteria on the OECT channel.

2. Results and Discussion
2.1. Characterization of the OECT

The single-well OECT was fabricated by spin-coating PEDOT:PSS solution on a glass patterned with ITO, whereby the ITO strips served as the source and the drain, respectively (Figure 1). A Pt gate electrode was used instead of an Ag/AgCl electrode since silver is known for its antimicrobial effect. By glueing a glass ring on the PEDOT:PSS coated glass, a well with a working volume of 1 mL was generated. To support bacterial growth but still keep the analyte solution defined and simple, a working volume of 1 mL was generated. To facilitate comparison, we normalized the data by assigning 0% to the smallest value of each measurement and 100% to the highest value. At V(s) > 0, the relative change of ISD of samples containing bacteria is higher than that of M9 medium only (Figure 2h). At VG +0.5 V, the ISD of samples containing bacteria is bigger for samples containing bacteria than for M9 medium only. This indicates that VG +0.5 V is suitable to operate the OECT also from a bacterial detection perspective (Figure 2i).

Collectively, both AVG(csgD) and ISD(csgD) appear to be reliable indicators of bacterial presence on the device and are independent of biofilm formation. However, quantification and real-time monitoring of bacterial growth based on AVG(csgD) may be difficult since the exhibited shifts varied and the resolution of the method is low. By contrast, changes in ISD(csgD) assume continuous values and therefore represent a promising strategy for electrochemical monitoring of bacterial growth on the OECT.

2.2. Monitoring Bacterial Growth on the OECT

Knowing that the ISD(csgD) at VG +0.5 V is bigger when bacteria are present in the M9 medium, we set out to determine if

Figure 1. Scheme of the PEDOT:PSS based single-well OECT.
we can monitor bacterial growth at higher temporal resolution. To determine the background signal, M9 medium was added to the single-well OECT and the signal was measured at different time intervals for ≥12 h. At t = 0, the medium was inoculated with S. Enteritidis WT or ΔcsgD and the signal was measured every hour for 24 h. For each measurement, ≥3 pulses were applied and the third was used for quantification (Figure 3a,b; Figure S3, Supporting Information). The initial $I_{SD}$ decreased slowly but steadily and no substantial change in the rate of its decrease was observed after the inoculation with bacteria (Figure S4a,b, Supporting Information). We suggest that the decrease in $I_{SD}$ can be attributed to the current drift, [28,39,40] most likely caused by environmental oxidation or conformational changes in the polymer matrix.[1] While we consider it unlikely, we cannot exclude the possibility that the applied $V_{SD}$ also contributes to the drift. In contrast with the initial $I_{SD}$, the Δ$I_{SD}$ was relatively stable before but increased after the inoculation with the WT bacteria (Figure S4c, Supporting Information).

The same was true for the ΔcsgD strain (Figure S4d, Supporting Information), indicating that the process is most likely not biofilm-related.

The absolute values of $I_{SD}$ and Δ$I_{SD}$ varied between experiments, which we attributed to device variability (e.g., different channel conductance leading to different initial $I_{SD}$). To account for this, the OECT modulation can be presented as |Δ$I_{SD}$/I_{SD}|.[16,41] In our experiments, the |Δ$I_{SD}$/I_{SD}| increased over time for both strains and no differences were observed between the strains (Figure 3c,d, Figure S4e,f, Supporting Information). The shape of the plotted data resembled that of a turbidimetric growth curve. By contrast, when the M9 medium was applied on the OECT, no increase in Δ$I_{SD}$ or |Δ$I_{SD}$/I_{SD}| was observed (Figure S5, Supporting Information), confirming that the changes we observed after inoculation were due to the growth of bacteria. It is known that the ionic strength of bacterial cultures increases during growth.[5,42,43] Glucose metabolized via oxidative phosphorylation yields CO$_2$, which forms carbonic

Figure 2. Electrochemical characterization of the single-well OECT. a–f) Transfer curves of OECTs recorded at (a–c) 0 h and (d–f) 24 h of incubation at 28 °C. OECTs contained (a,d) M9 medium without bacteria, or M9 cultures of S. Enteritidis (b,e) WT and (c,f) ΔcsgD. Circles show drain current ($I_{SD}$, left Y-axis), crosses show transconductance ($g_m$, right Y-axis). Symbols represent the mean of technical replicates, error bars show ± SD. Representative graphs of n = 3 biological replicates are shown. See Figure S1 of the Supporting Information for remaining experiments. g–i) Values for M9 medium (gray symbols), S. Enteritidis WT (black symbols), and S. Enteritidis ΔcsgD (clear symbols) recorded at t = 0 h subtracted from the corresponding values at t = 24 h for g) $V_G$ at maximum transconductance ($g_m$, max), h) normalized $I_{SD}$ for each $V_{C}$ and i) the $I_{SD}$ recorded at $V_{C}$ = +0.5 V. Symbols show mean ± SD of (g,i) technical or (h) biological replicates, error bars show ± SD.
acid when dissolved in water. Fermentation of glucose yields ethanol, acetic, formic, lactic, and succinic acid. We suggest that as all these products are charged, they increase the ionic strength and conductivity of the medium. It has been previously shown that OECTs can function as ion sensors since the $I_{SD}$ at a given $V_G$ decreases when the ion concentration increases. We, therefore, suggest that the OECT measures charged species secreted by metabolically active bacteria. It is known that several bacteria secrete redox active species and our group previously showed that *Salmonella* spp. can reduce PEDOT:PSS. Due to the more negative bias applied to the drain compared to the gate, it is unlikely that oxidation of reduced compounds would occur on the channel. It may, however, occur on the positively biased Pt gate, leading to a higher $I_{G}$ and $\Delta I_{SD}$.

To determine whether the increase in bacterial density and the OECT signal correlate, we performed turbidimetric growth curves by measuring the absorbance at 600 nm ($A_{600}$) of *S. Enteritidis* WT and ΔcsgD in M9 medium at 28 °C. Our data show that both strains reached a stationary phase between 12 and 16 h of growth, at $A_{600}$ ≈ 0.6 (Figure 4a,d). Deletion of csgD did not affect the growth rate.

When plotting the $|\Delta I_{SD}/I_{SD}|$ (from Figure 3) against the $A_{600}$, we observed a linear correlation between $A_{600}$ and $|\Delta I_{SD}/I_{SD}|$ throughout the exponential growth phase, until $A_{600}$ ≈ 0.6, when the cultures reached the early stationary phase (Figure 4b,e). The correlation disappeared, however, when the cultures entered the stationary phase at $A_{600} > 0.6$. Thereafter, the $|\Delta I_{SD}/I_{SD}|$ kept on increasing while $A_{600}$ remained stable or showed a slight decrease. The same trend was observed for both strains, indicating that ECM formation is not responsible for the observed pattern. We suggest that the lack of correlation between the two variables in the stationary phase can be explained by the fact that while bacteria in the stationary phase do not multiply, they remain metabolically active.

Presuming that the signal primarily originates from charged metabolic products, we hypothesized that the physical presence of bacterial cells would not be necessary to generate a positive signal. To test this, we collected supernatants from bacterial cultures grown statically at 28 °C in M9 minimal medium. After filtration, the supernatants were applied in the single-well OECT. We observed that the $|\Delta I_{SD}/I_{SD}|$ correlated with the incubation time for both *S. Enteritidis* strains (Figure 4c,f; Figure S6, Supporting Information), thus confirming our hypothesis. In an attempt to benchmark results from our OECT recordings to electrochemical impedance spectroscopy, we measured impedance spectra of the supernatants collected at 0 and 24 h. We did not observe any differences in overall impedance in any of the samples (Figure S7, Supporting Information), possibly because the changes in solution resistance are too small to be detected by this method.

### 2.3. SEM of Bacteria in the Single-Well OECT

To obtain a visual representation of the microenvironment on the PEDOT:PSS channel in the single-well OECT, we applied SEM. Following 24 h incubation of *S. Enteritidis* WT and ΔcsgD,
the OECTs were prepared for SEM. To avoid bias toward strongly adherent cells, we carefully replaced the medium with the fixative solution with no washing step. SEM of the WT showed bacterial cells distributed across the entire channel surface, and occasional bacterial aggregates were observed (Figure 5a,b). At higher magnification, ECM of the bacterial aggregates as well as rare filamentous bacteria was observed. The ΔcsgD strain showed an even distribution of cells on the surface, which were less clumped compared to the WT (Figure 5c,d). High magnification images revealed an expected lack of ECM. The topography of the polymer structure, illustrating its curvature, can be seen in Figure 5d. In summary, the SEM images are in agreement with results obtained by biofilm quantification using crystal violet (Figure S2, Supporting Information),

Figure 4. The single-well OECT signal reflects bacterial growth and metabolism. a,d) $A_{600}$ of separate static cultures of S. Enteritidis (a) WT and (d) ΔcsgD incubated at 28 °C. Each replicate of $n = 3$ is shown. b,e) $|ΔI_{SD}/I_{SD}|$ (as shown in Figure 3) versus $A_{600}$ (as shown in (a) and (d)) for S. Enteritidis b) WT and e) ΔcsgD. c,f) $|ΔI_{SD}/I_{SD}|$ for filtered supernatants collected from cultures of S. Enteritidis (c) WT and (f) ΔcsgD following 0, 4, 8, 12, 16, 20, and 24 h incubation at 28 °C. Data were background corrected by subtracting the value at $t = 0$ h from all values. Symbols show mean values of $n = 2$.

Figure 5. Visualization of bacteria on the PEDOT:PSS channel in the single-well OECT. SEM-SE images of S. Enteritidis (a,b) WT and (c,d) ΔcsgD grown in M9 medium in the single-well OECT for 24 h at 28 °C. (a–d) Each panel shows images of the same area at lower (left) and higher (right) magnification. Scale bars: 10 µm (a–d; left image), 0.5 µm (a,b; right image) and 1 µm (c,d; right image).
showing ECM formation predominantly by S. Enteritidis WT. The amount of ECM is relatively scarce and does not cover the polymer surface, which explains why we observe similar OECT responses of the WT and mutant strain.

3. Conclusion
This work demonstrates the use of a single-well OECT to monitor bacterial growth in real-time. Simple device design enables facile fabrication and avoids the use of recognition elements. We suggest that the key to the OECT response is an increase of charged species in bacterial culture produced as a consequence of bacterial metabolism. This makes the device generic, most likely applicable to be used for many different bacterial species. This method complements commonly used turbidimetry as it may provide more information on the metabolically active but nondividing bacteria, such as those in the stationary phase. We found that ECM production did not affect the OECT signal, however, this conclusion can currently not be extended beyond the strains and medium used in this study. SEM images have shown that ECM formation on the PEDOT:PSS channel was scarce, and it remains to be elucidated whether more abundant ECM production would lead to impaired ion diffusion to the channel, thereby altering the OECT signal.

Compared to optical recordings, electrochemical methods have the advantage of not requiring the analyte solutions to be transparent. Moreover, the OECT readout is based on the relative increase of the signal over time, which in theory means that no “blank” control is needed. We consider these advantages important for applications such as quality control in industrial settings as well as for diagnostic purposes. Collectively, we see our work on the single-well OECT as a proof-of-principle, opening for exciting applications to be developed in the future.

4. Experimental Section

Chemicals: Chemicals were obtained from Sigma-Aldrich (Sweden) unless otherwise stated. Phosphate buffer saline (PBS) pH 7.4 (Medicago, Sweden) was prepared in ultrapure water and autoclaved before use.

Bacterial Strains, Media, and Growth Conditions: S. Enteritidis 393d and the isogenic mutant ΔcsgD were kept as glycerol stocks at −80 °C and plated on Tryptic Soy Agar (Sigma-Aldrich, Sweden) plates before use. M9 minimal medium with 0.4% glucose (referred to as M9 medium) was prepared as previously described.[50] The glucose concentration was increased to 4% when indicated. Overnight cultures were prepared by inoculating a single colony from the plate in 5 mL M9 medium and culturing for ≈12 h at 37 °C, 160 rpm.

To determine the growth rate of bacteria in static conditions, the overnight cultures were diluted 1:100 in M9 medium and aliquoted in 15 mL tubes, 2 mL in each tube. The tubes were incubated at 28 °C and each hour one tube was taken out and the absorbance at 600 nm (A600) was measured. The experiment was performed on a single occasion, in biological triplicates, i.e., with three cultures each of S. Enteritidis WT and ΔcsgD. One measurement for one biological replicate of S. Enteritidis ΔcsgD was identified to be an outlier and excluded from the data.

For supernatant collection, the cultures were prepared as described above, only that 15 mL medium was placed in 50 mL tubes, the samples were collected every fourth hour and the sample collection was performed on two independent occasions. To obtain filtrates, bacterial cultures were centrifuged and the supernatants were filtered using 0.2 µm polyethersulfone filters (Sartstedt, Germany). The filtrates were stored at −20 °C and thawed before use.

Device Fabrication and Setup: PEDOT:PSS (Clevios PH1000, Heraeus, Germany) was added 2 v/v% glycerol and 0.25 v/v% 4-dodecylbenzenesulfonic acid, stirred for at least 30 min and filtered through a 5 µm polycylylene fluoride filter (Merck, Germany). It was then mixed thoroughly and sonicated for 60 min. Then, 1 v/v% (3-glycidyloxypropyl) trimethoxysilane was added to the solution that was mixed thoroughly. Glass patterned with ITO strips (15 Ohm sq−1) with dimensions 20 mm × 20 mm × 0.7 mm (XinYan Technology LTD, Hong Kong) were cleaned by 20 min successive sonication in acetone (VWR, Sweden), isopropanol (VWR, Sweden), and ultrapure water. Just before coating, glass slides were plasma cleaned for 30 s in Uvo Cleaner model 24 (Jelight, USA). Conducting polymer solution was spin-coated on cleaned patterned glass slides in two steps: 10 s at 500 rpm, followed by 45 s at 1500 rpm using WS-650-23 Spin Coater (Laurrel Technologies Corporation, USA). Coated patterned glass slides were baked for 90 min at 120 °C. Glass rings with 16 or 18 mm outer diameter, 1.2 mm wall thickness, and 10 mm height (Scientific Lab Glass AB, Sweden) were glued on the coated patterned glass using a 1:10 mixture of silicone elastomer and silicone elastomer curing agent (Dow Corning, USA), on a heating pad at 120 °C for ~5 min to generate a single-well OECT with a working volume of 1 mL. To expose the source and drain electrodes, the conducting polymer layer outside of the glass ring was scraped off with a razor blade. Fabricated devices were stored at room temperature, in dry and dark conditions. Before the experiment, the OECTs were placed in a humidified incubator keeping the temperature at 28 °C. The source and drain electrodes (i.e., ITO), contacted with tungsten needles (Quarter Research, USA), and the Pt wire gate electrode (Gamry, USA) were kept in place with XYZ-300 Test positioners (Quarter Research, USA) and connected to a SourceMeter (Keithley, USA).

Electrochemical Measurements: Single-well OECT devices were characterized using a SourceMeter (Keithley, USA) controlled with a customized LabVIEW program. Transfer curves of single-well OECTs containing 1 mL M9 medium ± 10 µL overnight culture of S. Enteritidis WT and ΔcsgD were determined at t = 0 h and t = 24 h of OECT incubation at 28 °C. Before the transfer curve measurement, the device was gated four times (VGC = ±0.5 V, VGC step = 0.5 V. VGO = −0.5 V) to validate its function and to depolarize the gate electrode. The transfer curves were recorded in technical quadruplicates, at VGO = −0.5 V and VGC ranging from −0.7 to +0.7 V in steps of 0.1 V. Transconductance was determined by calculating the first derivative of the drain current.

For continuous measurements, 1 mL of M9 medium was applied in the well and each device was stabilized by increasing VGC from 0.1 to 0.5 V. Thereafter, the device was operated at −0.5 VSD and +0.5 VGC. Gate pulses were 30 s long, ≥3 pulses were applied at each time point. The third pulse was always used for analysis. The signal was measured in irregular time intervals for >12 h, denoted as t = 0, to establish the baseline. At t = 0 h, M9 medium was inoculated with 10 µL overnight culture, and the OECT response was measured every hour for ≥24 h.

Electrochemical impedance spectroscopy was performed with Reference 600 Potentiostat (Gamry, USA) using a glassy carbon working electrode (CH Instruments, USA), Ag/AgCl reference electrode (CH Instruments, USA) and Pt counter electrode (CH Instruments, USA). The frequency range was 10−1–105 Hz, 10 points per decade, 10 mV ms. Each measurement was performed in technical triplicates.

Crystal Violet Assay: Crystal violet assay was performed as previously described,[51,52] with minor modifications. Overnight cultures were diluted 1:100 in M9 medium with 0.4 or 4% glucose. 3 × 1 mL of M9 medium (used as control) and of each diluted culture was pipetted into a 24 well plate (Corning Costar, USA), which were incubated for 24 h at 28 °C, then rinsed four times with distilled water and dried at 70 °C for ~40 min. Subsequently, 1 mL of 0.1% crystal violet was applied in each well and incubated for 15 min at room temperature. The dye was decanted
and the plates were rinsed four times with distilled water. After drying at 37 °C, 1 mL of 30% acetic acid was applied in each well and the plate was incubated for ~30 min at room temperature, shaking. Absorbance at 590 nm (A590) was measured in a Synergy MX Plate Reader (Biotek, USA).

**Scanning Electron Microscopy:** To prepare single-well OECTs for SEM, the medium was decanted, and the devices were stored in fixation solution (2.5% glutaraldehyde in PBS pH 7.4). Before imaging, the well was removed and the devices were soaked in ultrapure water for 2 min to remove the fixative, repeating the step three times. The samples were dried by incubation in 30%, 50%, 70%, and 95% ethanol for 10 min. This was followed by 2 × 15 min of dehydration in 99.5% ethanol. Then, the samples were placed in an automated critical point drying chamber at 8 °C, 50 bar, continuous tiring under CO2 atmosphere. This was followed by 2 × 15 min of dehydration in 99.5% ethanol. Then, the samples were sputtered with platinum (150–200 Å) to enhance the sample conductivity. Imaging was performed on Zeiss Ultra 55 scanning electron microscope, using a secondary electron detector, EHT 3 kV and 5–6 nm working distance.

**Data Visualization and Analysis:** Single-well OECT data analysis was performed with Python programming language using Jupyter notebooks,[33] Pandas,[34] and Plotly[35] packages. Prism 9 (GraphPad Software Inc, USA) was used to plot the data.

**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.

**Keywords**

bacterial growth, organic electrochemical transistors, real-time monitoring of bacterial growth, *Salmonella*

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