H₂O₂ plays a significant role in a range of physiological processes where it performs vital tasks in redox signaling. The sensitivity of many biological pathways to H₂O₂ opens up a unique direction in the development of bioelectronics devices to control levels of reactive-oxygen species (ROS). Here a microfabricated ROS modulation device that relies on controlled faradaic reactions is presented. A concentric pixel arrangement of a peroxide-evolving cathode surrounded by an anode ring which decomposes the peroxide, resulting in localized peroxide delivery is reported. The conducting polymer (poly(3,4-ethylenedioxythiophene) (PEDOT), is exploited as the cathode. PEDOT selectively catalyzes the oxygen reduction reaction resulting in the production of hydrogen peroxide (H₂O₂). Using electrochemical and optical assays, combined with modeling, the performance of the devices is benchmarked. The concentric pixels generate tunable gradients of peroxide and oxygen concentrations. The faradaic devices are prototyped by modulating human H₂O₂-sensitive Kv7.2/7.3 (M-type) channels expressed in a single-cell model (Xenopus laevis oocytes). The Kv7 ion channel family is responsible for regulating neuronal excitability in the heart, brain, and smooth muscles, making it an ideal platform for faradaic ROS stimulation. The results demonstrate the potential of PEDOT to act as an H₂O₂ delivery system, paving the way to ROS-based organic bioelectronics.

1. Introduction

Reactive oxygen species (ROS) regulate vital biological processes and are involved in essential signaling pathways during redox metabolism. While the majority of studies are dedicated to understanding their toxicity in connection with numerous medical conditions, for example, neurodegenerative diseases, atherosclerosis, or aging, an increasing number of reports support the notion that ROS exhibit important physiological roles in maintaining redox homeostasis. Especially with regard to hydrogen peroxide (H₂O₂) the research field of redox biology has gained valuable insight into ROS-mediated mechanisms that underlie a variety of functions ranging from immune response to angiogenesis. As early as 1970, pioneering work by Sies & Chance provided the first evidence that hydrogen peroxide is generated in aerobic biological cells. So far, several intracellular sites have been identified as physiological sources of H₂O₂, most notably the mitochondria and NADPH oxidase. Moreover, it is established that H₂O₂ is produced during oxidative protein folding and via peroxisomal enzymes. The most prominent role of H₂O₂ is known to be its contribution to redox signaling as a secondary messenger. The non-radical nature and longer half-life under physiological conditions allow H₂O₂ to act as a signaling molecule. The reversible oxidation of thiol groups belonging to deprotonated cysteine residues in proteins stands out among the several multifaceted redox reactions that can be initiated by H₂O₂ which in turn results in altered protein activity. These signaling
mechanisms have a major impact on cell proliferation, survival, and differentiation. These effects can proceed with even low H$_2$O$_2$ concentrations in the range of 1–10 nM. Here, the fine redox balance is maintained via scavenging systems (e.g., catalase) that remove excess amounts of ROS which otherwise would cause irreversible damage to nucleic acids, proteins, and lipids.[21]

In the past decades, H$_2$O$_2$ has also been gradually coming to the fore as an oxidative modulator of ion channels. Several more recent electrophysiological studies have revealed the ability of H$_2$O$_2$ to directly activate ion channels, including ATP-sensitive potassium (K$_{ATP}$) and transient receptor potential (TRP) channels.[22] In particular, the sensitivity of voltage-gated Kv7.2/7.3 channels toward oxidative modification has gained much attention since this type of potassium channel exerts crucial tasks related to neuronal activity.[22,23] Heterotetrameric Kv7.2/7.3 channels are encoded by KCNQ2 and KCNQ3 genes and control neuronal excitability in hippocampal as well as in dorsal root ganglion neurons by increasing the threshold for action potential firing.[24–26] The anti-excitatory role of Kv7.2/7.3 channels is largely due to the fact that subthreshold membrane voltages (∼−60 mV) are sufficient to activate Kv7.2/7.3, and that Kv7.2/7.3 channels remain in their activated state despite extended time of activation.[27,28] Consequently, Kv7.2/7.3 channels generate an outward potassium current, also referred to as M-current, which contributes to the negative resting membrane potential of neurons. M-current constitutes a photochemical production of peroxide/ROS by Antognazza and colleagues[38] as a reducing agent for in vivo electrical peroxide delivery. This work highlights the potential of PEDOT to act as faradaic delivery material for modulation of neuronal voltage-gated ion channels and thus takes a step forward in understanding and establishing faradaic stimulation mechanisms which will constitute the basis for future advancements of ROS-mediated neuromodulation.

2. Results and Discussion

2.1. Electrochemistry of Faradaic Pixels: The Balance between Oxygen and Hydrogen Peroxide

The faradaic peroxide delivery devices were designed using a concentric arrangement of a central cathode surrounded by an anode ring (Figure 1a). This way, H$_2$O$_2$ produced at the cathode will be spatially confined by the anode, as H$_2$O$_2$ which arrives at the anode will be readily oxidized back to O$_2$. It is critical to choose suitable cathode and anode materials. The first consideration is to select a cathode that reduces oxygen selectively to H$_2$O$_2$. The cathodic electrochemical reactions relevant in this work are shown in the following:

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \quad (1)$$
$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$
$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (3)$$
$$2H^+ + 2e^- \rightarrow H_2 \quad (4)$$

An ideal electrocatalyst for this application is one that enables Equation (1), while not favoring the remaining Equations (2)–(4). For this purpose we use the conducting polymer formulation PEDOT with poly(styrene sulfonate), shortened as PEDOT:PSS, or PEDOT in this paper. PEDOT is known to be a redox mediator for selective two-electron reduction of oxygen to hydrogen.
Figure 1. Faradaic pixels electrically modulate hydrogen peroxide and oxygen concentrations. a) Schematic and photo of the device, featuring a circular PEDOT:PSS cathode in the center, surrounded by a palladium anode. The cathode produces H₂O₂ via the 2-electron oxygen reduction reaction, while the anode completes the DC electrochemical circuit by the anodic reactions of water oxidation and peroxide oxidation. b) Cyclic voltammetry recordings of PEDOT:PSS versus bare Titanium in pH 7.4 electrolyte with different O₂ contents: air saturated, 0% (purged with N₂), and 100% (purged with O₂). The volume of solution that was placed on top of the active area (6.15 mm²) was 30 μL. Larger volumes were initially purged with either O₂ or N₂ and subsequently an aliquot of 30 μL used for recordings. c) Cyclic voltammetry (4 cycles) measurement of the palladium counter electrode before and after addition of 10 mm H₂O₂. d) Fluorescent images of the electrolyte droplet containing Amplex UltraRed reagent placed on top of the PEDOT:PSS pixel. The PEDOT:PSS pixel device was operated for 10 min and changes in the fluorescent signal of the Amplex UltraRed reagent were recorded over time. Increase in fluorescence intensity (greyscale) was evaluated by plotting the intensity over time. e) Mean quantities (nmol, left axis) of H₂O₂ produced by the device obtained via the HRP-TMB assay after running the PEDOT faradaic pixel device galvanostatically at 10 μA cm⁻² for 5, 10, 15 and 20 min (± SD, n = 3–5, number of measured samples in total = 5). Faradaic efficiency is calculated at each time point, and is plotted on the right axis. Theoretical values indicate a situation with 100% faradaic yield. f) Digital camera imaging was used to calculate the distance, d = 250, 500, 750 μm, of the amperometric sensor from the surface of the pixel. Distance was calibrated using the known thickness of the microscope slide as a standard. g,h) Mean H₂O₂ and O₂ concentration traces acquired via local amperometric recordings using H₂O₂ and O₂ sensors 250, 500, and 750 μm above PEDOT:PSS film surface for a run time of 10 min. i,j) Mean H₂O₂ and O₂ concentration traces measured 250 μm above the device surface during alternating on/off time periods with a duration of 5 min (on)/5.35 min (off), respectively (± SD, n = 3–6, number of measured samples in total: 4).

Remarkably, compared to other 2-electron catalysts, PEDOT allows for the net accumulation of peroxide because the further reduction (Equation (3)) is kinetically hindered. The four-electron oxygen reduction reaction (Equation (2)) does not occur at any observable rate. The two-electron hydrogen-evolution reaction (Equation (4)) can happen on PEDOT, however only at very low pH values.[40] This reaction is not efficient at pH values exceeding 3. Therefore, under neutral pH conditions, Equation (1) can be expected to predominate on PEDOT. Facile processability and good biocompatibility make PEDOT a logical choice for the peroxide-evolving cathode element in this device.

The anode material should satisfy the conditions of good stability under positive polarization, insensitivity to corrosion by peroxide, and the ability to catalyze both peroxide oxidation (Equation (5)) and water oxidation (Equation (6)):

\[ H₂O₂ → O₂+2H⁺+2e⁻ \]  \hspace{1cm} (5)

\[ 2H₂O → O₂+4H⁺+4e⁻ \]  \hspace{1cm} (6)

\[ 2H₂O₂ → O₂+2H₂O \]  \hspace{1cm} (7)

These conditions are met by many of the group VIII transition elements, the so-called platinum-like metals such as palladium, platinum, ruthenium, or iridium.[41] Based on easy processability, high stability, and relatively lowest cost, we chose palladium as the anodic electrode.[42] Another property of the platinum metals is that they efficiently catalyze the nonfaradaic disproportionation reaction of hydrogen peroxide to water and oxygen (Equation (7)).[43] This is an important aspect of the faradaic pixel design, as this means that the palladium ring will decompose peroxide regardless of whether current is on or off.

The complete design of the faradaic pixel therefore consists of a central PEDOT cathode, surrounded by a palladium ring (Figure 1a). We used titanium as a suitable underlying conductor for both cathode and anode. Titanium is chosen due to its relative
To quantify the faradaic evolution of hydrogen peroxide, we employed two separate techniques which give different important information. The first is the HRP/tetramethylbenzidine (HRP/TMB) optical absorption assay[45] (Figure 1e). The second is amperometric sensing with peroxide-specific and oxygen-specific microsensor probes (Figure 1f–j). The HRP/TMB assay is used to quantify the average concentration of H$_2$O$_2$ in the volume of electrolyte. This can therefore be used to compute the faradaic efficiency (FE) of peroxide production by using the total charge that is passed through the electrochemical circuit. For accurate measurement of FE, experiments are performed with the palladium electrode covered with a rubber block, and a platinum counter electrode placed in a separate anode compartment separated from the cathode chamber by an agarose salt bridge. This is critical to confine the produced H$_2$O$_2$ to the cathode chamber and prevent its diffusion and subsequent oxidation at the anode. This would otherwise yield an underestimated FE. Figure 1e shows the amount of peroxide produced over a 20-min long galvanostatic (−1.9 μA = 10 μA cm$^{-2}$) experiment, as well as the corresponding faradaic yield. As oxygen is consumed and [H$_2$O$_2$] grows, the faradaic yield is 76% ± 2% over 20 min. The remaining charge is consumed by two side processes, Equations (3) and (4): peroxide reduction and hydrogen evolution, respectively.

The HRP/TMB assay allows quantitative faradaic yield calculations, this method cannot probe if there are gradients in [H$_2$O$_2$] over the electrolyte volume. In reality, the concentration of peroxide should vary as a function of time and position relative to the cathode. It is logical that the concentration of H$_2$O$_2$ should be higher closer to the cathode, especially at the start of the experiment. For this reason, amperometric microsensors are very useful to probe the concentration in a position-dependent way. We used two types of amperometric sensors: one for peroxide and the other for dissolved oxygen. We placed these sensors at fixed positions above the peroxide-evolving cathode (Figure 1f), and ran galvanostatic experiments with the concentric PEDOT:PSS versus palladium design. Measuring at different distances reveals a clear gradient in evolved peroxide and consumed O$_2$ (Figure 1g,i). Over the course of a 10-min galvanostatic experiment with 1.9 μA of current, large differences in [H$_2$O$_2$]/[O$_2$] are apparent. At a distance 250 μm above the center of the cathode, peroxide concentration rises to 150 μm, while simultaneously the concentration of dissolved oxygen drops from an initial equilibrium value to 260 μm down to 140 μm. The ratio [H$_2$O$_2$]/[O$_2$] is 0.57 at this position. Measuring at points further away from the cathode surface, the ratio [H$_2$O$_2$]/[O$_2$] declines. At a height of 750 μm, the ratio is 0.22. We performed cyclic measurements with current set to 1.9 μA, 10 min on, followed by 10 min off (Figure 1h). These cycles show a reproducible peroxide concentration increase followed by decline when current is shut off. The corresponding oxygen consumption and recovery curves (Figure 1j) represent a mirror image to the peroxide measurements. Peroxide declines when current is off due to its disproportionation at the palladium electrode (Equation (7)), as well as the minor contribution of consumption by the amperometric sensor current (nA level currents). In this pixel configuration, the peroxide concentration has a half-life of 2.3 min (Figure S2, Supporting Information). Oxygen concentrations recover when current is shut off, as [O$_2$] is replenished by dissolution and diffusion of O$_2$ from the air around the electrolyte drop. As expected, placing the peroxide sensor over
the palladium electrode yields much lower peroxide values, by about an order of magnitude (Figure S3, Supporting Information). Meanwhile, oxygen concentrations measured above palladium increase above baseline values during oxidation of H$_2$O$_2$ to O$_2$ (Figure S4, Supporting Information). The factors at play in the differential gradients of O$_2$ and H$_2$O$_2$ are complex, as they will depend on many variables such as current, faradaic efficiency, diffusion coefficients, and temperature. The interplay of these factors is best evaluated using computational methods which will be addressed in the following section. From these experimental measurements, however, the picture that emerges is that peroxide and oxygen concentrations can be varied significantly, with the largest changes being localized at the PEDOT cathode. The palladium anode functions as a guard electrode, blocking peroxide diffusion and maintaining relatively constant levels of dissolved oxygen. PEDOT is therefore a good catalyst for accumulation of peroxide, in accordance with earlier reports.[39] We performed also control experiments with bare titanium as a cathode, finding that equilibrium concentrations of peroxide are about an order of magnitude lower than those formed over PEDOT electrodes (Figure S5, Supporting Information).

2.2. Computational Modeling of Oxygen and Peroxide Gradients

The relative concentrations of peroxide and O$_2$ will depend on time, position, and the respective electrochemical and disproportionation reactions. The concentrations can be computed over time and position using diffusion coefficients of H$_2$O$_2$ and dissolved O$_2$ and the geometry of the cathode, anode, and water droplet. Based on this, we constructed a finite element model, with parameters and boundary conditions given in Figure 2a. We perform calculations on the “bare” device geometry, as well as considering the addition of the X. laevis oocyte that is used in the electrophysiology experiments in Section 2.3. Several values, such as the initial concentration of oxygen $c_0$, production rate $R$, and temperature of the system $T$, are given. The initial equilibrium concentration of dissolved oxygen in the electrolyte solution was measured to be $c_0 = 265 \mu$m. A production rate of H$_2$O$_2$ is dependent on the current applied to the PEDOT cathode, which in all experiments was $1.9 \mu$A. The temperature of the solution was set to 23 °C. These parameters were used to reproduce the experimental setup in simulations. The simulation process was divided into two parts, namely the production stage that goes for 600 s and the consumption stage for the next 600 s when produced peroxide is freely diffusing over the solution droplet and can be decomposed at the palladium surface. 3D concentration profiles of H$_2$O$_2$ are depicted in Figure 2b and Video S1, Supporting Information. An increase of H$_2$O$_2$ concentration is observed on the time scale 600 s with a maximum value near the PEDOT surface and gradient decrease with the distance due to diffusion. During the next 600 s, no current is applied to PEDOT, and therefore no peroxide is produced. Over this time, the available H$_2$O$_2$ continues diffusing further into the droplet medium. Simultaneously, oxygen will diffuse down its gradient toward the PEDOT to compensate for its consumption. The net decrease in total peroxide concentration in the droplet occurs in two possible ways: consumption on the palladium electrode or evaporation from the droplet surface. To better understand the process, the influence of each of the critical parameters: T, $c_0$, and R were studied individually and compared with the data obtained in experiment (Figure 2c–e). The temperature of the solution strongly influences the diffusion coefficients of the diluted species. Higher temperature means higher diffusion coefficients and faster spreading of the peroxide across the droplet. The change of the temperature from 23 to 37 °C leads to the increase of $D_{H_2O_2}$ and $D_{O_2}$ of $\approx 35\%$. At 37 °C, the recorded $c_{H_2O_2}$ at the end of the production stage (600 s) is almost the same, except the narrow $\mu$m region in the vicinity of the PEDOT surface. As for oxygen, the impact of increased temperature is more pronounced. Compensation of oxygen losses happens noticeably faster. (Figure 2c).

The initial content of O$_2$ ($c_0$) in the droplet plays a crucial role in peroxide production. O$_2$ serves as a “fuel” for the reaction, and its supply at the reaction area (PEDOT surface) defines H$_2$O$_2$ flux.Reducing the $c_0$ from 265 to 100 $\mu$m results in the decrease of H$_2$O$_2$ production roughly by three times (Figure 2d). While the production in the first seconds is still relatively high, from 200 s all of the dissolved oxygen near PEDOT becomes fully depleted. Under these conditions of O$_2$ starvation, peroxide production will cease. When this happens under galvanostatic conditions, the current will be primarily consumed via the reduction of peroxide to water (Equation (2)).

A similar situation takes place when the peroxide production rate is increased instead of decreasing the oxygen content. Production of peroxide can be boosted by the increase of current applied to PEDOT. Assuming doubled current compared to the experimental conditions (3.8 $\mu$A), peroxide production significantly increased during the first 100 s simultaneously with the consumption of all available dissolved oxygen near PEDOT (Figure 2e). After the first 100 s, the faradaic efficiency of the process drops from 95% to 20%, and H$_2$O$_2$ production therefore drastically decreases. As a result, despite a rapid start, the resulting concentrations of peroxide after 600 s of production are almost the same for $I = 1.9 \mu$A or $I = 3.8 \mu$A. Simultaneously, the oocyte would suffer heavy oxygen starvation due to oxygen depletion at the PEDOT surface. Even at a distance of 1 mm from the pixel, $c_0$ is twice smaller than its initial value. It is important to note that due to the fact that both H$_2$O$_2$ and O$_2$ are uncharged small molecules, they diffuse readily through cell membranes. When the oocyte is included in the model (as shown in Figure 2a), its effects on the H$_2$O$_2$ and O$_2$ gradients are negligible (Figure S6, Supporting Information), that is, both molecules freely move through the oocyte as through electrolyte alone.

The obtained simulation indicates how peroxide production by a PEDOT pixel proceeds according to three interconnected factors: production rate, availability of dissolved oxygen, and diffusion coefficients of the diluted species. The formation of the peroxide on the surface of PEDOT is followed by its diffusion further into the droplet medium with an apparent gradient in concentration. Simultaneously, depletion of oxygen happens near the surface of the pixel, which invokes diffusion of the available dissolved oxygen from the droplet toward PEDOT. The calculated oxygen and peroxide concentrations over time closely match what is found experimentally (Figure 1g, h) using microamperometric probes. For the model to match experiment in terms of peroxide accumulation over cycles, the boundary condition at the edge of the droplet assumes peroxide concentration is zero outside the droplet (Figure S7, Supporting Information). This indicates that
Figure 2. Finite element modeling illuminates the interplay of O2 and H2O2 gradients. a) 2D slice of the 3D model, used during the simulations of H2O2 and O2 diffusion with given boundary conditions and regions of production/consumption of diffused species. The geometry reproduces that of the electrophysiology experiments on oocytes (brown sphere in the center). b) 3D concentration profiles of H2O2 at different times from 0 to 1200 s (Current is turned on during the first 600 s, then switched off). c–e) Calculated [H2O2](toward) and [O2] (bottom row) at a different height from the center of the PEDOT pixel: 1 μm, 0.5 mm, 1 mm. The calculations closely follow the microamperometry experimental setup. Each calculation shows the effect of a different critical parameter: c) temperature, d) initial O2 concentration, and e) applied current. At a distance of 1 μm, O2 concentration drops to values below the measurable threshold, indicated by the flattening of the black lines.
the model is in fact accurate and can be applied to understand how oxygen and peroxide concentrations can vary under different conditions and device geometries. If the goal is to maximize the production of H$_2$O$_2$, the supply of sufficient dissolved O$_2$ is critical. In the case of a too large production rate, a rapid decrease of the faradaic efficiency occurs, accompanied by oxygen depletion. The effect of droplet volume loss due to evaporation can also be easily accounted for in the model. The results (Figure S8, Supporting Information) demonstrate that marked effects to oxygen and peroxide gradients are not present until evaporation loss is around 50% of the original droplet volume.

### 2.3. Electrochemical H$_2$O$_2$ Delivery Facilitates Opening of the Human Heteromeric Kv7.2/7.3 M-Channel

Following characterization of H$_2$O$_2$ generation at the PEDOT/electrolyte interface, we set our attention to using these devices to modify electrophysiological properties of ROS-sensitive voltage-gated ion channels. For this purpose, we chose *X. laevis* oocytes to monitor the faradaic effects of H$_2$O$_2$ at a single cell level. *X. laevis* oocytes are a well-established single-cell electrophysiology model system, known for its robustness, large size (1 mm ø), and ability to reliably express a chosen type of ion channel.\[^{46}\] To test the PEDOT-mediated H$_2$O$_2$ delivery we expressed human heteromeric Kv7.2/7.3 M-channels in *X. laevis* oocytes. The sensitivity of Kv7.2, Kv7.4, and Kv7.5 channels to H$_2$O$_2$, and the lack thereof for Kv7.1 and Kv7.3 channels, has been established previously in a Chinese hamster ovary cell model conducted by Gamper et al.\[^{23}\] This paper reports that currents generated both by Kv7.2 and Kv7.2/7.3 are augmented by application of extracellular H$_2$O$_2$. The proposed mechanism behind the peroxide sensitivity is semi-reversible oxidative modification of three cysteine residues in the intracellular S2-S3 linker.

All experiments on oocytes were conducted using the conventional two-electrode voltage-clamp technique (TEVC) in the arrangement as shown in Figure 2a (experimental setup photos shown in Figure S9, Supporting Information). In the voltage protocol used to measure K$^+$ currents, the holding potential was set to $-100$ mV. Depolarizing test steps between $-100$ and $+40$ mV (2 s, $10$ mV increments) were used to open the channel, followed by a step back to $-30$ mV (1 s) to measure tail currents (Figure 3a inset). Prior to testing the PEDOT pixel device, it was essential to confirm that the Kv7.2/7.3 channels expressed in *X. laevis* oocytes were sensitive to H$_2$O$_2$ (Figure 3a–c). An initial control recording before perfusion of H$_2$O$_2$ was performed to quantify the current generated under control conditions. This was followed by perfusion experiments in which different concentrations of H$_2$O$_2$ (5, 50, and 300 μM) were consecutively introduced into the bath chamber. After perfusing each concentration of H$_2$O$_2$, we performed TEVC recordings using the same voltage protocol as in the control experiment. Depolarizing the membrane potential resulted in an opening of the voltage-gated Kv7.2/7.3 channels and subsequent outward flow of potassium ions, which was recorded as a positive current that reaches saturation (steady-state) 2 s after onset of depolarizing voltage pulses above $-30$ mV. Figure 3a depicts representative current traces generated by stepping to a test voltage of $+40$ mV. A clear augmentation of the steady-state current ($I_{ss}$) at $+40$ mV was visible with increasing H$_2$O$_2$ concentrations, 300 μM H$_2$O$_2$ increased the current by $2.26 \pm 0.06$-fold (Figure 3a,b, Table 1). In the concentration-response curve the maximum increase, given as the ratio $I_{ss,max}/I_{ss,control}$, is $2.41 \pm 0.07$, and the half maximal concentration $37.4 \pm 6.9$ μM (Figure 3b, Table 1). To determine if the H$_2$O$_2$-induced augmentation at $+40$ mV was a result of an increase in the maximal conductance ($G_{MAX}$) alone or accompanied by a shift in the voltage dependence of channel activation, we quantified the instantaneous tail current (when stepping to $-30$ mV after each test voltage, Figure 3a) under control conditions, and after perfusion with H$_2$O$_2$. Figure 3c shows representative tail currents ($I_{tail}$) plotted versus the preceding test voltage, normalized to the control, clearly demonstrating the ability of H$_2$O$_2$ to increase the maximal conductance ($G_{MAX}$) in a concentration-dependent manner, while having no effect on the voltage dependence for channel activation ($\Delta V_{50}$, Figure 3c, Table 1). Taken together, this demonstrates that Kv7.2/7.3 channels expressed in *X. laevis* oocytes are susceptible to H$_2$O$_2$ delivered via perfusion, with H$_2$O$_2$ augmenting the current generated by Kv7.2/7.3 channels by increasing the conductance.

After having established a reliable oocyte model, expressing H$_2$O$_2$-sensitive Kv7.2/7.3 channels, we proceeded with probing the electrochemical delivery of H$_2$O$_2$ with the PEDOT faradaic pixel device. We measured oocytes using TEVC in the configuration as shown in Figure 2a, with the cell positioned in the center of the PEDOT cathode. TEVC recordings were carried out while simultaneously operating the faradaic pixel under galvanostatic conditions. Adapting the cycle protocol (on, off) introduced previously (Figure 1i), we ran four cycles with the PEDOT device and measured steady-state and tail currents during each off-period using the voltage protocol established in initial perfusion recordings (Figure S10). Contrary to the perfusion experiments, where the [H$_2$O$_2$] is assumed to be homogenous throughout the measurement solution, from the experiments discussed in Sections 2.1 and 2.2, we know that a gradient of [H$_2$O$_2$] will exist in the experiment. The bottom part of the oocyte that is closest to the PEDOT film surface will experience the highest amount of H$_2$O$_2$ while the top half is exposed to lower H$_2$O$_2$ amounts. Therefore, for all experiments with the faradaic pixel (Figure 3d–f), instead of concentration [H$_2$O$_2$], we refer to molar amount of H$_2$O$_2$ (nmol), and in terms of the electrical charge (μC) that has passed through the PEDOT during active operation. As in the prior perfusion experiments, we started measurements with a control recording of the oocyte without H$_2$O$_2$. Not only does a control measurement serve as point of reference but is also a good indicator for the quality of Kv7.2/7.3 channel expression and an assurance that the PEDOT device does not harm the oocyte cell membrane.

Figure 3d shows representative steady-state and tail current traces recorded at $+40$ and $-30$ mV, respectively. A clear rise in $I_{ss}$ at $+40$ mV (Figure 3d,e, Table 1), and $G_{MAX}$ (Figure 3f, Table 1), is apparent with increasing amount of H$_2$O$_2$ that is being delivered to the oocyte after each on cycle with the PEDOT device: 2.19, 4.53, 6.53 and 9.17 nmol of H$_2$O$_2$ for cycle 1–4, respectively (Figure 3d,e, Table 1). After the fourth cycle the steady-state current increased by $2.10 \pm 0.07$-fold, similar to what is expected with around 100 μM H$_2$O$_2$ in the perfusion experiments (Figure 3b). Since the oocytes become less stable over time (e.g., because of membrane leakage) we did not run more than 4 cycles with the PEDOT device (≈45 min), which...
Figure 3. Faradaic delivery of H₂O₂ modifies currents in H₂O₂-sensitive Kv7.2/7.3 ion channels. Panels a–c): Effect of H₂O₂ delivered via perfusion on the Kv7.2/7.3 M-channel. a) Representative current traces for one oocyte before (control, light orange) and after perfusing H₂O₂ with concentrations of 5, 50, and 300 μM (indicated by darker color gradient, as in (c) at following test voltages +40 mV (steady-state), −30 mV (tail). Inset: voltage protocol showing 3 s-long test pulses applied throughout all TEVC experiments. b) Relative change in steady-state current at +40 mV ($I_{ss, test}/I_{ss, ctrl}$, Table 1) for 5, 50, and 300 μM H₂O₂ (mean ± SEM, n = 3). Concentration-response curve fitted using Equation (8); $A = 2.41 ± 0.07$, $C_{50} = 37.39 ± 6.86$ μM. c) Representative normalized $I_{tail}$ for one oocyte, curve fitted with Equation (9). Panels d–f): Activation of Kv7.2/7.3 channels upon electrochemical H₂O₂ delivery with PEDOT faradaic pixel devices. d) Representative steady-state (+40 mV) and tail current (−30 mV) current traces measured in a single oocyte placed on top of the PEDOT cathode before (control) and after exposure to 2.19, 4.53, 6.53, and 9.17 nmol H₂O₂ (indicated by darker color gradient, as in (f). e) Relative change in steady-state current ratios at +40 mV ($I_{ss, test}/I_{ss, ctrl}$, Table 1) (mean ± SEM, n = 11). Concentration-response curve fitted using Equation (8), although a saturation in the curve is not reached. f) Normalized $I_{tail}$ plotted against preceding test voltage, data fitted with Equation (9) (mean ± SEM, n = 11, in total 10 different PEDOT faradaic pixel devices were tested).

explains why a saturation in the concentration-response curve is not reached (Figure 3e). We also could see a small but statistically significant shift in the voltage dependence of activation (−2–3 mV), but independent of the number of PEDOT device cycles (Figure 3f, Table 1). The electrochemical H₂O₂ delivery via the PEDOT device thus clearly increases the maximal conductance of the Kv7.2/7.3 channel, similar to H₂O₂ in the perfusion experiments in the micromolar range.

In order to confirm that the Kv7.2/7.3 channel activation is in fact being caused by electrochemically-produced H₂O₂, we conducted time-match controls (Figure 4). These controls are obtained by doing TEVC measurements of oocytes expressing Kv7.2/7.3 using the same cycle interval as before, but without operating the PEDOT device (Figure S10, Supporting Information). After 30–40 min (cycle 3–4), $I_{ss}$ at +40 mV and $G_{MAX}$ increased marginally, ≈1.2–1.25-fold (Figure 4a,c, Table 1), while no significant effect was seen for the voltage dependence of channel activation ($ΔV_{50}$, Figure 4c, Table 1). Such a small rise in current amplitude is normal during longer oocyte recordings. The augmentation is significantly lower compared to when the PEDOT device is running (Figure 4f), thus suggesting the electrochemically-produced H₂O₂ is responsible for effect.

As a further validation of the peroxide-induced current enhancement, we also tested if the current increase for the Kv7.2/7.3 channel can be attributed to the oxidative modification of triple cysteine residues in the Kv7.2 subunit.[23] Guided by experiments showing that a triplet of cysteines in the intracellular S2-S3 linker is responsible for H₂O₂ effects in Kv7.4, we constructed a Kv7.2 mutant with the corresponding cysteines mutated to alanines (i.e., Kv7.2/C150A/C151A/C152A). The lack of the triple cysteine residues also made the Kv7.2 channel insensitive to peroxide, 500 μM H₂O₂ had no effect on $I_{ss}$ at +40 mV, $G_{MAX}$, or $ΔV_{50}$ in the perfusion experiments, even after 20 min incubation time (Figure 4d, Table 1). When the faradaic pixel device was tested on the Kv7.2 mutant channel (same 4 cycles as before, Figure S10, Supporting Information) there was no significant increase in $I_{ss}$ at +40 mV or $G_{MAX}$ (Figure 4a,e,f, Table 1). The voltage dependence of channel activation was also unchanged (Figure 4e, Table 1). This suggests that the faradaic pixel is acting upon the electrophysiology of the Kv7.2/7.3 channels.
primarily through oxidation of the triple cysteine residues in the Kv7.2 subunit by electrochemical delivery of H$_2$O$_2$.

Altogether, the oocyte experiments show that the faradaic pixel device can modify the behavior of human neuronal Kv7.2/7.3 channels via electrochemical dosing of peroxide, demonstrating the potential of the faradaic pixel device to control H$_2$O$_2$-sensitive physiological responses.

### 3. Discussion and Conclusions

O$_2$ reduction is the key reaction in cellular respiration. Respiration pathways also produce ROS as byproducts: peroxide, superoxide, and hydroxyl radicals. The balance of these species is critical to homeostasis, as high levels can result in toxic effects, while lower concentrations mediate important signaling pathways. Artificial manipulation of oxygen chemistry in biological systems using electrical devices could be a powerful tool for basic research and eventually biomedical devices. In this work, we report an electrochemical device fabricated using conventional microfabrication techniques which is capable of tuning O$_2$/H$_2$O$_2$ levels in a localized way. Due to the manufacturing method, these devices can be obtained in any desired size or layout. For example, the faradaic pixel can be made in larger configurations to target tissues or organs, or alternatively shrinked down into microelectrode array formats appropriate for experiments with cultured neurons or brain slices. Regardless of size scale, the device acts as an H$_2$O$_2$ delivery electrode that consumes dissolved O$_2$ as a “fuel.” The electrochemical circuit is completed by a counter electrode, which is made out of palladium in order to efficiently eliminate the peroxide which is produced. This design can be used to ensure the localization of peroxide delivery. Device size and shape effects can be anticipated using the finite element model we have herein introduced. To visualize how the concept scales, the peroxide gradient for cathodes of size 5–0.5 mm diameter is shown in Figure S11, Supporting Information. Shrinking down the size of the peroxide-generating cathode leads to the same peroxide concentration at the cathode's surface, but a much sharper spatial gradient. However, the effect of oxygen depletion is much less pronounced for smaller cathodes. The device centers around the redox properties of organic electronic materials. Based on their unique electrocatalytic and photocatalytic properties combined with relative stability and biocompatibility, organic semiconductors seem uniquely poised to provide tunable ROS production for biological applications. Several organic small-molecules as well as polymers have shown the ability to photo(electro)catalytically reduce oxygen to...
Figure 4. Increased potassium currents are primarily caused by delivered H$_2$O$_2$ acting on a cysteine triplet in Kv7.2. Panels (b–e) are fitted with sigmoidal Boltzmann function (Equation (9)). Panel a) concentration-response plots for electrochemical H$_2$O$_2$ delivery, showing the results for steady-state currents at +40 mV in Kv7.2/C150A/C151A/C152A and a time-matched control with device switched off for Kv7.2/7.3 (mean ± SEM, n = 3 (Kv7.2/C150A/C151A/C152A), n = 3 (Kv7.2/7.3 time-match)). Panels b,c) comparison between TEVC recordings of Kv7.2/7.3 after delivering 9.17 nmol of H$_2$O$_2$ via the PEDOT cathode (panel b, ± SEM, n = 11; includes same data as in panel 3(f) and during a 45 min-long time-match control without H$_2$O$_2$ where the cathode is not operated (panel c, mean ± SEM, n = 3). Panel d) TEVC recordings of H$_2$O$_2$-insensitive Kv7.2/C150A/C151A/C152A after perfusing 500 μM H$_2$O$_2$ for 10 and 20 min (mean ± SEM, n = 3). Panel e) exposure of Kv7.2/C150A/C151A/C152A to 9.17 nmol H$_2$O$_2$ via PEDOT cathode (mean ± SEM, n = 3). Panel f) statistical analysis and comparison of relative steady-state I$_{\text{ss,test}}$/I$_{\text{ss,ctrl}}$ obtained for Kv7.2/7.3 and Kv7.2/C150A/C151A/C152A upon delivery of 9.17 nmol H$_2$O$_2$ by the faradaic pixel device. Middle bar “Time-match Kv7.2/7.3” shows relative steady-state I$_{\text{ss,test}}$/I$_{\text{ss,ctrl}}$ of oocytes recorded without operating the PEDOT cathode in time-match experiments, (mean ± SEM, n = 3–11, asterisk: p < 0.0001 (****), p < 0.005 (**)).
enable less-invasive deployment of H$_2$O$_2$ delivery in electrophysiological experiments, it will be possible to drive such devices using photovoltaics. The concept of photofaradaic pixels was introduced by our group recently for the H$_2$O$_2$/glucose redox pair,[36] and this can be adapted to the protocols discussed in this paper. We believe the results we have presented should stimulate research into reactive-oxygen species mediated neuromodulation. In parallel, the findings should also encourage materials scientists and electrochemists to tap into the vast body of knowledge with oxygen redox chemistry to develop high-performance devices for the manipulation of oxygen in physiological conditions.

4. Experimental Section

Materials and Device Fabrication: All chemicals were purchased from Sigma Aldrich unless otherwise noted. Device samples were fabricated on glass microscope slides 3 x 1. This following stepwise sonication in acetone (10 min), isopropanol (10 min), 2% Hellmanex III detergent (10 min), and deionized water (DI) (30 min) the glass slides were dried under N$_2$ and additionally used throughout all measurements in this study were adjusted to pH 7. The imaging was conducted with an Eclipse Ti Nikon fluorescence microscope in a darkroom using a TRITC filter. Prior to operation, the PEDOT:PSS pixel was covered with a 200 μL droplet of 1K electrolyte solution and 10 μL of the Ampex UltraRed/HRP mixture added to the droplet.

HRP-TMB UV–Vis Assay: The average H$_2$O$_2$ concentration generated above the central PEDOT:PSS pixel was determined spectrophotometrically via an HRP-TMB assay with a Synergy H1 Microplate reader (BioTek Instruments, Inc.). The HRP-TMB solution used for the assay was freshly prepared by mixing 2 μL HRP solution (0.75 mg mL$^{-1}$) and 5 μL 3,3′,5,5′-tetramethylbenzidine (TMB, 10 mg mL$^{-1}$) solution in 993 μL disodium phosphate (Na$_2$HPO$_4$) (0.2 m) citric acid (0.1 m) buffer solution (diluted 1:4 with DI water and filtered, pH 5.6). The 5 mm wide circular PEDOT:PSS/Ti film was operated galvanostatically at −1.9 mV by placing a 50 μL droplet of 1K electrolyte solution on top of the film surface. The droplet was connected to a platinum mesh counter electrode via a freshly prepared agarose salt bridge. After operating the PEDOT:PSS device for 5, 10, 15, and 20 min, a 35 μL aliquot was taken out of the 50 μL droplet and added to 265 μL HRP-TMB assay solution and mixed thoroughly in a 96 well-microplate. The colorimetric change of the solution upon the HRP-catalyzed oxidation of TMB by H$_2$O$_2$ was measured at 530 nm. In order to quantify the H$_2$O$_2$ concentration a 5-point calibration curve was established by measuring the colorimetric changes in 5 mixtures of H$_2$O$_2$ (1 mm stock solution in 1K) with HRP-TMB assay solution with known H$_2$O$_2$ concentrations: 0, 10, 20, 30, and 40 μM.

Amperometric Sensing of Peroxide and Oxygen: The quantification of H$_2$O$_2$ and O$_2$ concentrations locally at the 1K electrolyte/PEDOT:PSS interface was performed with a 4-Channel Free Radical Analyzer (World Precision Instruments, WPI) equipped with 2 mm wide H$_2$O$_2$ (ISO-HPO-2, WPI) and O$_2$ (ISO-OXY-2, WPI) sensors (polarization voltages: $V_{O_2} = 950$ mV and $V_{H_2O_2} = 750$ mV). Lab-Trax4/16 (WPI) and LabScribe software (version 4.31) were used for recording the sensor current. O$_2$ and H$_2$O$_2$ concentrations were determined separately. The sensors were mounted onto a stereotaxic frame which allowed accurate positioning of the sensor tip above the PBS bath and the solution was continuously stirred. 165, 330, 750, and 750 μL of a 1 mM H$_2$O$_2$ stock solution in DI water was sequentially added to the PBS bath and changes in the sensor current were recorded continuously. A 2-point calibration (0% and air-saturated) was used to quantify the O$_2$ concentration. Here, the O$_2$ sensor was inserted into a commercially available calibration bottle (WPI) filled with DI water that was continuously stirred. First, the sensor current in air-saturated DI water was measured before the content of the bottle was purged with N$_2$. 1K solutions used throughout all measurements in this study were adjusted to room temperature (±23 °C) over the course of several days. Contrary to HRP-TMB experiments, the whole device architecture including the palladium counter electrode was covered with 200 μL 1K solution during all amperometric recordings.

Electrophysiology Measurements with X. laevis Oocytes: X. laevis oocytes were either obtained via surgery and prepared for injections at Linköping University (approved by the Linköping Animal Care and Use Committee (Permit #1941)) or purchased from Ecocyte Bioscience. The X. leavis
Oocytes were injected 3–5 days prior to recording with 50 nL RNA containing 2.5 ng Kv7.2 and 2.5 ng Kv7.3 cRNAs (Kv7.2: GenBank accession no. NM_004518, Kv7.3: GenBank accession no. NM_004519, 1:1 molar ratio) and incubated at 8 °C. To ensure sufficient ion channel expression, the injected oocytes were stored at 16 °C overnight 1 day before experiments and used for recordings that were performed at room temperature. During perfusion experiments a pump (model ISM97D: Labdor Lab AB) was used, with a perfusion rate of 0.5 mL min⁻¹. The Kv7.2/C150A/C151A/C152A mutant was constructed using site-directed mutagenesis (QuickChange II XL with 10 XL Gold cells, Agilent) with correct sequence alteration ensured by sequencing at the Core Facility at Linköping University. X. laevis oocytes were injected 3–5 days prior to recording with 25 ng Kv7.2/C150A/C151A/C152A and incubated at 8 °C. All two-electrode voltage clamp recordings (TEVC) were conducted with a whole-cell amplifier (CA-18 Dagan Corporation). 1K electrolyte was used throughout all measurements as extracellular medium. During measurements with the PEDOT:PSS faradic pixel device both the Pd counter electrode as well as the PEDOT:PSS film were covered with a 200 μL droplet of 1K solution. The droplet was connected to both TEVC reference electrodes via a freshly prepared agarose salt bridge.

In the voltage protocol, the holding voltage was set to −100 mV. A multi-step voltage protocol with test voltages ranging from −100 to +40 mV (10 mV increments, 2 s duration) was used to generate steady-state currents, followed by a subsequent test voltage at −30 mV to generate tail currents (1 s duration). The sweep-to-sweep interval was 15 s, and the whole protocol took ≈3.5 min. Data were sampled at 5 kHz and filtered at 500 Hz.

After control recordings, the PEDOT:PSS faradic pixel device was turned on in 4 on/off cycles (5 min ON, 5.5 min OFF, Figure S10, Supporting Information). To assess the effect on Kv7 channel opening, the voltage protocol shown in Figure S10, Supporting Information. To assess the effect on Kv7 channel opening, the voltage protocol shown in Figure S10, Supporting Information. Overall, the diffusion process in oocyte membrane data were analyzed with Clampfit 11.1.0.23, Matlab R2019a, and GraphPad Prism 9.

Analysis of Currents: Potassium currents were leak-subtracted and steady-state currents (I_{ss}) at +40 mV were quantified at the end of the voltage sweep. The relative change was determined as the ratio of I_{ss, test}/I_{ss, ctrl}, and the concentration-response curve was plotted using the following equation:

$$\frac{I_{ss, test}}{I_{ss, ctrl}} = \frac{A}{1 + \frac{C^2}{C_0}}$$

(8)

where I_{ss, test}/I_{ss, ctrl} is the relative change in the steady-state current, A the amplitude of the curve, C_0 the concentration at which half-maximal response occurs, and C the concentration (in μM or nM).

Instantaneous tail current (I_{tail}) was measured (after onset of the −30 mV step) and plotted against the preceding test voltage. Data were normalized to the control by dividing the data set by the current value of the control at +40 mV. To generate the conductance versus voltage (G(V)) curve in Figures 3c,f, 4b-e the following Boltzmann function was fitted to the normalized I_{tail} data:

$$G(V) = G_{min} + (G_{MAX} - G_{MIN}) / \left\{ 1 + \exp \left[ \frac{(V_{SO} - V)}{s} \right] \right\}$$

(9)

where G_{MIN} is the normalized minimal conductance, G_{MAX} the normalized maximal conductance, V_{SO} the midpoint (i.e., the voltage needed to reach half the maximal conductance determined from the fit), V the test voltage, and s the slope of the curve (shared between test and control curve).

The relative change in G_{MAX} was calculated as the ratio G_{MAX,test}/G_{MAX,ctrl} and the shift in the voltage dependence of activation (∆V_{SO}) was calculated as V_{SO, test} − V_{SO, ctrl} from the Boltzmann fit.

Statistical Analysis of Electrophysiological Data: Mean values presented in Section 2.3 are expressed as mean ± SEM if not stated otherwise. A one-sample t-test was used to compare to a hypothetical value of 1 (for relative I_{tail} or G_{MAX} or 0 (for ∆V_{SO}). When comparing groups in Figure 4f, one-way ANOVA with Dunnett’s multiple comparison test was used. Effects were considered statistically significant if P < 0.05.

Computational Methods: Simulations were conducted using the finite element method, implemented in the COMSOL software package, version 5.5 (https://www.comsol.com/product-download).

Schematic 2D slice of the modeled system can be found in Figure 2a, and the full 3D shape is in Figure 2b. The geometry of the simulation model was chosen to replicate experimental setup and consisted of three main regions: PEDOT pixel and palladium circle, covered with a water droplet. The parameters and sizes of model regions are depicted in Figure 2a.

There were two variables in the system: concentration of dissolved oxygen (O_2) and concentration of peroxide (H_2O_2). At a starting point, there was no H_2O_2 in the system, while the initial value of O_2 was c_0, where c_0 = 265 μM was an equilibrium concentration of dissolved oxygen measured experimentally. The following equations express the diffusion of O_2 and H_2O_2 through the PEDOT:PSS film with were sample data at 5 kHz and filtered at 500 Hz.
concentration of $O_2$ required for maximal faradic efficiency. Reactions on the palladium electrode were reversed and lead to the decomposition of peroxide. If $H_2O_2$ molecules reach the Pd regions, they will be converted into dissolved oxygen with unity efficiency.

Switching between the production and consumption of molecules was implemented with the "events" interface and happened in two stages. Each of stages has its own boundary conditions on PEDOT:PSS surface (Figure 2a). The first stage happens when current was applied to PEDOT pixel (from 0 s to 600 s). During this stage, peroxide was produced and oxygen was consumed near the PEDOT surface simultaneously with the reversed reaction on palladium surface. The second stage happens when the current on the pixel was switched off (no inward or outward fluxes on PEDOT:PSS surface) with the reversed reaction on Pd still taking place (from 600 s to 1200 s). All boundary conditions, used during the implementation are defined in Figure 2a. The three simulation probe sites for detection of $c_{H_2O_2}$ and $c_{O_2}$ were located on top of the PEDOT pixel center at the distance of 1 μm, 0.5 mm, and 1.0 mm, covering all height of an oocyte starting from its lowest point.

**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.

**Author Contributions**

S.I.L. and E.D.G. conceived the project idea. O.S.A. performed all device fabrication, electrochemical measurements, and assays, and most electrophysiological recordings. S.I.L. conducted original peroxide perfusion recordings. I.S. performed the calculations with finite-element models. M.S.E. and M.J. contributed to sample preparation, experimental methods and design, and data analysis. The project was led and supervised by I.Z., S.I.L. and E.D.G. The manuscript was written and figures prepared with input from all coauthors.

**Data Availability Statement**

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

**Keywords**

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