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Controlling the Resting Membrane Potential of Cells with Conducting Polymer Microwires

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Graphical abstract

Conducting polymer microwires were used to control the resting membrane potential of *E. coli* cells. It is expected that these microwires will provide a new, non-invasive, cellular-scale, tool for the control of resting membrane potential with high spatial precision.

Supporting Information

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

Keywords

conducting polymers; PEDOT:PSS; membrane potential; depolarization

All cells have a resting membrane potential resulting from an ion gradient across the plasma membrane. The resting membrane potential of cells is tightly coupled to regeneration and differentiation. The ability to control this parameter provides the opportunity for both biomedical advances and the probing of fundamental bioelectric pathways. The use of PEDOT:PSS conducting polymer microwires to depolarize cells was tested using *E. coli* cells loaded with a fluorescent dye that is pumped out of the cells in response to depolarization, a more positive membrane potential. Fluorescence imaging of the cells in response to a conducting polymer microwire-applied voltage confirms depolarization and shows that the rate of depolarization is a function of the applied voltage and frequency. Microwire activity does not damage the cells, demonstrated with a propidium iodide assay of membrane integrity. The conducting polymer microwires do not penetrate the cell, or even need to contact the cell, they only need to generate a minimum electric field, controlled by the placement of the wires. It is expected that these microwires will provide a new, non-invasive, cellular-scale, tool for the control of resting membrane potential with high spatial precision.

1. Introduction

All cells maintain a -10 mV to -100 mV electrical potential across the plasma membrane driven by an ion gradient.^[1] This resting membrane potential is essential to cellular proliferation and differentiation.^[2–5] Non-proliferating cells, such as neurons, are hyperpolarized, with more negative membrane potentials. Actively proliferating cells, such as undifferentiated stem cells, are depolarized, with potentials closer to 0 mV. Membrane potential is tightly coupled to tissue regeneration. Depolarization induces mitosis and DNA synthesis in neurons.^[6–8] Tadpoles depend on a sequence of depolarization and

hyperpolarization to regenerate tails.^[9–11] Frogs can be induced to grow extra eyes through localized hyperpolarization.^[12] Depolarization maintains the plasticity of stem cells and hyperpolarization leads to differentiation.^[13, 14] And while much attention has been paid to the action potentials of excitable cells such as neurons and heart cells, perhaps more important is this resting membrane potential, which is present in every cell.

Established chemical and biological tools for controlling membrane potential include extracellular salts (potassium, choline chloride),^[15, 16] exogenous bacterial ion channels (gramicidin A, valinomycin),^[7, 17] toxins (ouabain, palytoxin),^[8, 10, 18, 19] genetic expression of ion channels,^[9, 20] and pharmacological agents that target specific ion channels.^[20, 21] More novel methods include the use of nanoparticles to depolarize cells and optogenetic approaches for light-based control of ion fluxes.^[22–26] These are all population-level reagents, affecting all treated cells. For applications requiring spatial resolution, new approaches capable of addressing single cells or even sub-cellular regions are necessary. For example, the ability to perturb the membrane potential of single cells would provide an experimental test of bioelectric communication pathways.^[27, 28]

We have recently demonstrated the use of poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) conducting polymer wires for a biological application, controlling the localization of proteins in solution.^[29] The PEDOT:PSS wires have tunable diameters (150 nm – 4.5 μ m), lengths ranging from nanometers to millimeters, and conductivities of ~ 20 S/cm.^[29–33] Physically, the conducting polymer wires function as a cellular-sized “knob” to control the ion concentration at the plasma membrane. The wires are non-invasive. They do not penetrate the cell or even need to contact the cell, they only need to generate a minimum electric field, controlled by the placement of the wires.

To test the use of these conducting polymer wires to control the resting membrane potential of cells, *E. coli* cells were loaded with Rhodamine 123, a “Nernstian” fluorophore used to measure bacterial membrane potential.^[34–36] Following treatment with a bacterial ionophore, gramicidin S, decreased membrane potential is indicated by decreased staining of bacteria, as the fluorophore is unable to accumulate in the cells. Most recently, rhodamine dyes have been used with *E. coli* to understand the relationship between membrane potential and flagella rotation and to probe the activity of a voltage-sensitive fluorescent protein.^[37, 38] Fluorescence intensity of individual cells was measured as a function of applied voltage parameters. Changes in fluorescence intensity were compared to the electric field felt by each cell, calculated using a COMSOL model. Importantly, a cell health assay to measure membrane integrity shows no cellular damage in response to the applied voltage of the conducting polymer wires.

2. Results and Discussion

2.1. Modulation of the Resting Membrane Potential of Bacterial Cells

PEDOT:PSS conducting polymer wires have been used previously to modulate the localization of charged species in solution.^[29] To determine if this ability to control ion concentrations could also be used to depolarize cells, we tested the use of the wires with *E. coli* loaded with Rhodamine 123, a green fluorophore (Excite: 507 nm, Emit: 529 nm) that is

pumped out of bacterial cells in response to depolarization,^[37, 39] a more positive membrane potential. Rhoadmine 123-loaded *E. coli* were attached to a poly-L-lysine modified glass coverslip and the intensity of individual cells was monitored with fluorescence microscopy (Olympus IX71, 100× objective, FITC filter). Micromanipulators were used to bring a conducting polymer microwire (1 μm diameter, 50 μm length) and a gold counter-electrode to the coverslip (Figure 1A–C). The microwire and gold counter-electrode were separated by 50 μm during cellular modulation. Fluorescence microscopy images were then recorded (60 ms exposure, 1 frame per second, iXon EMCCD, Andor) as a voltage was applied through the conducting polymer wire, starting at 5 s. Unless otherwise noted, a ± 2.5 V biphasic square wave with a frequency of 0.5 Hz (50% duty cycle) was used. To quantify the change in fluorescence intensity with respect to applied voltage, ten cells were randomly selected (Figure 1C) and their normalized intensity, measured with ImageJ, was plotted as a function of time (Figure 1D). Over 120 s, we observe a decrease of $\sim 50\%$ in the fluorescence intensity of individual cells in response to the applied pulse.

To ensure that the decrease in fluorescence intensity was not due to photobleaching, the same experiment was carried out in the absence of an applied voltage (Figure 1D), showing minimal decrease in fluorescence intensity. Similarly, to confirm that the change in intensity is due to the conducting polymer microwire, rather than the gold electrode to which it is attached, we carried out control experiments with gold electrodes in the absence of microwires (Figure S1). These experiments showed only a minor (less than 10%) decrease in fluorescence intensity for cells >10 μm away from the gold electrode surface, which was used as a minimum distance for the microwire analysis.

2.2. Effect of Pulse Parameters on Depolarization

Our initial experiments demonstrated that conducting polymer microwires could be used to depolarize bacteria (Figure 1). We next probed the relationship between depolarization and the pulse parameters used for depolarization by varying pulse frequency, voltage, and shape (Figure 2 and Figure S2). We first varied the pulse frequency from 0.05 Hz to 2.5 Hz while keeping the voltage constant at ± 2.5 V (Figure 2A). Data is plotted for an average of 10 cells at each frequency and fit to a double exponential decay. A plot of the decay rate, using the first term in the double exponential, shows an increase in depolarization for increased frequencies (Figure 2B). Similarly, experiments using a constant frequency (0.5 Hz) and varied voltage (± 0.05 V to ± 3.00 V) showed increased depolarization at increasing voltages (Figure 2C and D).

A biphasic square wave was used in all experiments described above (Figure 1 and 2). With a single step pulse (held at $+2.5$ V or -2.5 V) depolarization is not observed (Figure S2A and S2B). In comparison, a square wave between 0 and $+2.5$ V resulted in depolarization similar to a biphasic square wave (Figure S2C and S2D). This suggests that in the absence of a changing pulse (biphasic or square wave) the cells are able to re-establish an ion gradient, preventing depolarization. This is supported by the frequency-dependent depolarization (Figure 2A and B), which shows increased frequencies lead to greater depolarization.

2.3. Cell Health is Maintained After Microwire-Induced Depolarization

To determine if microwire activity had any negative effect on the health of the cells, a propidium iodide (PI) assay was used following microwire activity (Figure 3). PI is a cell impermeant fluorogenic molecule that can only enter *E. coli* with damaged cell walls. It then becomes fluorescent (Excite: 535 nm, Emit: 617 nm) upon intercalation with DNA. Cells were incubated with PI (50 μ M, 30 min) and then a ± 4 V, 1 Hz pulse was applied to the cells for 1200 seconds as a test of extreme pulse conditions. PI internalization was not observed (Figure 3B), demonstrating that cell health is maintained. As a positive control for cell death, cells were heated to 80 $^{\circ}$ C for 15 minutes (Figure 3C and D).

2.4. Cellular Response as a Function of Electric Field

Initial experiments pointed towards depolarization as a function of cellular position relative to the microwire (Figure 1D). To probe this directly, the experimental parameters (microwire, applied voltage, imaging media) were modelled using COMSOL to determine the electric field at each cell (Figure 4A). In these initial simulations, the cells were assumed to have the same conductivity as the surrounding medium and did not affect the electric field. The conducting polymer microwires were treated as smooth cylinders planar to the cells. Although electric double layer formation during voltage steps could change the electric field over time, stationary electric currents were used to determine the electric field in this initial model.

The change in fluorescence for individual cells, a measure of depolarization, was compared to the electric field calculated at that individual cell (Figure 4B), showing a linear correlation. Assuming that there is no coordination between ion channels, the side of the cell closer to the conducting polymer wire is depolarized and, relative to this depolarization, the opposite side is hyperpolarized. In these experiments, only depolarization is detected as Rhodamine 123 is pumped out of the cells. Additionally, the *E. coli* are relatively small on the scale of the conducting polymer wire. In future experiments, larger cells, such as mammalian epithelial cells, and a combination of voltage-sensitive fluorophores, could be used to provide spatial resolution of polarization.

3. Conclusion

All cell types have a resting membrane potential and the ability to control this parameter will provide new directions in fundamental studies of bioelectricity, as well as regenerative medicine. We demonstrate the use of a new, non-invasive, tool, conducting polymer microwires, to control the membrane potential of individual *E. coli* cells (Figure 1), quantified by the fluorescent intensity of a “Nernstian” dye localized in the cells. The level of depolarization is controlled by the frequency and voltage of the applied pulse (Figure 2). Cell wall integrity, characterized by PI internalization, is not disrupted by the microwires (Figure 3), even at high (± 4 V) voltages for extended periods (1200 s). COMSOL modelling shows that the extent of depolarization is correlated with the electric field felt by each cell (Figure 4), pointing to a high level of spatial control. While these initial experiments use *E. coli* as a model system, we expect the use of the conducting polymer microwires to depolarize eukaryotic cells will be similar. For these larger cells, microwires could be used

to depolarize single cells or sub-cellular regions providing spatial patterning of membrane potential. Overall, controlling the resting membrane potential of *E. coli* with conducting polymer wires is a first step towards new bioelectric studies and applications.

4. Experimental Section

Cell culture

E. coli (initially cultured from One Shot TOP10, #C404010, Invitrogen, Carlsbad, CA), were grown in LB broth (#611875000, ACROS Organics/Thermo Fisher, Waltham, MA) at 37°C in a shaking incubator (Innova 44, Hauppauge, NY). Cells were grown overnight and diluted 1:50 for use in conducting polymer wire experiments. Cells were imaged in minimal media (M9 salts: 64 g Na₂HPO₄·7H₂O, 15 g KH₂PO₄, 2.5 g NaCl, 5.0 g NH₄Cl).

To measure cell viability, we used internalization of propidium iodide (PI) as an indicator of membrane integrity (50 µM PI in minimal media, #P1304MP, Molecular Probes/Invitrogen, Eugene, OR). As a positive control for dead cells, *E. coli* were incubated at 80°C for 15 min.

Poly-L-Lysine (PLL) immobilization of *E. coli*

Custom-made two-chambered dishes were used for depolarization experiments to allow electrochemical synthesis of the conducting polymer wires in one chamber and cellular experiments in the other chamber. To prevent movement of the *E. coli* during imaging, the cell culture half of the dishes were functionalized with PLL. The dishes were first plasma cleaned (1 min, 18 W, air, Harrick Plasma, Ithaca, NY) and then a PLL solution (0.01%, 100 µL, #P4832, Sigma-Aldrich, St. Louis, MO) was added to the glass surface. The dish with PLL solution was rocked at room temperature for 3 hr and then multiple rinses (1 mL, ×5) with MilliQ water were used to remove unbound PLL.

Bacterial staining with Rhodamine 123

E. coli are impermeant to Rhodamine 123 requiring permeabilization of the cell wall for delivery. Suspensions (1 mL) of *E. coli* were centrifuged (4000 rpm, 5 min, ×3) in LB broth to exchange media. The pelleted cells were mixed with EGTA (1 mM, #324626, Calbiochem, La Jolla, CA) for a final volume of 1 mL, vortexed, and incubated at 37°C for 1 min. The EGTA chelates calcium leading to permeabilization of the bacterial cell wall. To remove EGTA, the cell suspension was centrifuged (4000 rpm, 5 min, ×3) and washed with LB broth. Rhodamine 123 (10 µg mL⁻¹, #R302, Thermo Fisher) was introduced into the cells by incubating the cell suspension, in LB broth, with Rhodamine 123 at 37°C for 30 min. Free dye was removed by centrifugation (4000 rpm, 5 min, ×3) and resuspension in LB broth.

Electrochemical synthesis of PEDOT:PSS conducting polymer wires

Directed electrochemical nanowire assembly, described previously,^[29–31] was used to synthesize the conducting polymer wires. In brief, the PEDOT:PSS microwires were synthesized in an aqueous solution containing 3,4-ethylenedioxythiophene monomer (EDOT, 10 mM, 483028, Sigma-Aldrich) and polystyrene sulfonate (PSS, 20 mM, 243051, Sigma-Aldrich) as a counterion. Polymerization was carried out using a function generator

(33120A, Agilent, Santa Clara, CA) supplying an alternating, square-wave voltage (3 kHz) across two sharp, custom-made gold electrodes. Electrodes were spaced 50 μm apart (tip-to-tip) during the electrochemical synthesis. The gap between the counter-electrode and the end of the growing conducting polymer wire was held constant by manually adjusting one of the micromanipulators during synthesis. Wire diameter and length was measured using brightfield microscopy (Olympus IX71, 100 $\times$ objective, Andor iXon EMCCD camera).

Depolarization of *E. coli* with conducting polymer wires

After synthesizing the PEDOT:PSS wires, the initial EDOT and PSS solution was exchanged for ultrapure deionized water (EASYPure II, Barnstead) and minimal media. Rhodamine-loaded *E. coli* were then added to the solution for a final volume of 1.5 mL. The cells were allowed to settle onto the PLL-functionalized coverslip for 30 min. A function generator was used to deliver a voltage pulse (50 mV-4 V, 0.05 Hz-2.5 Hz, 50% duty cycle) to the conducting polymer wire, which remains attached to the gold electrode used for synthesis. The conducting polymer wire and the gold counter-electrode were separated by 50 μm during experiments to minimize contribution from the gold electrodes.

Depolarization, observed as a decrease in the intracellular concentration of Rhodamine 123, was monitored by fluorescence microscopy. Images were collected using an epi-fluorescence microscope (Olympus IX71, 100 $\times$ objective, 1.30 N.A., oil immersion, FITC filter) and an EMCCD (1 frame per second, 60 ms exposure, iXon, Andor, Belfast, Ireland). Profile plots (ImageJ, NIH)^[40] of fluorescence intensity for ten randomly selected bacteria, were used to quantify depolarization. To quantify the rate of depolarization, the decrease in fluorescence was fit to a double exponential with t_0 defined as the time at which a 1% decrease in signal was observed.

Calculation of electric field at individual cells

To determine the correlation between the strength of the electric field the *E. coli* experience and their change in fluorescence intensity, the experimental parameters were modelled using COMSOL (COMSOL Multiphysics modelling software, Palo Alto, CA). An image of the *E. coli* and conducting polymer wires was analyzed (ImageJ) to determine the coordinates of the bacteria relative to the conducting polymer wire. The wire was then modelled using COMSOL's Cone, Bezier Polygon, and Sweep tools. COMSOL's stationary electric currents module was used with the far edge of the wire set to the voltage applied by the function generator and the far edge of the gold counter-electrode set to ground. The electric conductivities were set to 45,600,000 S/m (COMSOL value), 3,000 S/m (based on two-point probe of similar conducting polymer wires), and 1.5 S/m (based on conductivity of phosphate buffered saline) for the gold counter-electrode, the PEDOT:PSS wires, and the cell culture medium, respectively. The relative permittivity values for gold and PEDOT:PSS were set to 1, and the value for the PBS was set to 80. A MATLAB (MathWorks, Natick, MA) script was written to take the electric field values as a function of position and map these values onto the fluorescence microscopy images of *E. coli*. The change in fluorescence (final-initial/maximum value) was then plotted against the electric field calculated at the individual bacteria cells. A linear fit was used to determine correlations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Modulation of *E. coli* with microwires. A) Fluorescence microscopy image of *E. coli* (green) prior to an applied voltage. B) Following an applied voltage (± 2.5 V, 0.5 Hz, 500 ms). C) A brightfield image of the same cells shows the position of wires. D) Fluorescence intensity of individual cells as a function of time. The number corresponds to the cells in (C). The photobleaching control (black) is the average of 10 cells. Error bars show standard deviation. All data was normalized by dividing each value by the maximum observed value within that data set.

Figure 2.

Effect of pulse parameters on depolarization. A) Fluorescence intensity as a function of time with an applied voltage of ± 2.5 V and frequencies from 0.05 Hz to 2.5 Hz (50% duty cycle). Each data set is the average of 10 cells. B) Rate of decrease in fluorescence intensity (data from 2A fit to a double exponential, first term shows initial decay) as a function of frequency. C) Fluorescence intensity as a function of time with a frequency of 0.5 Hz and an applied voltage of ± 0.05 V to ± 3 V. Each data set is the average of 10 cells. D) Rate of decrease in fluorescence intensity (data from 2C fit to a double exponential, first term is plotted) as a function of voltage.

Figure 3.

Cell health measured by propidium iodide (PI) internalization. A) Brightfield image prior to an applied voltage. B) A ± 4 V, 1 Hz, 50% duty cycle pulse was applied for 1200 seconds as an example of extreme applied voltage. The fluorescence microscopy image shows no PI internalization, indicating intact cell walls. C) Brightfield image of the positive control. Cells were heated to 80 °C for 15 min. D) The corresponding fluorescence microscopy image shows PI (red) internalized into the heat-killed cells.

Figure 4. Cellular response as a function of electric field. A) Microscopy image of wires and cells overlaid with the image of the COMSOL model of the electric field. B) Change in fluorescence intensity as a function of electric field (V/mm) felt by each cell numbered in (A). This data is representative of three distinct experiments with $n=20$ cells in each data set.