Influencing Biological Behavior Using Electrical Signals in Single Cells

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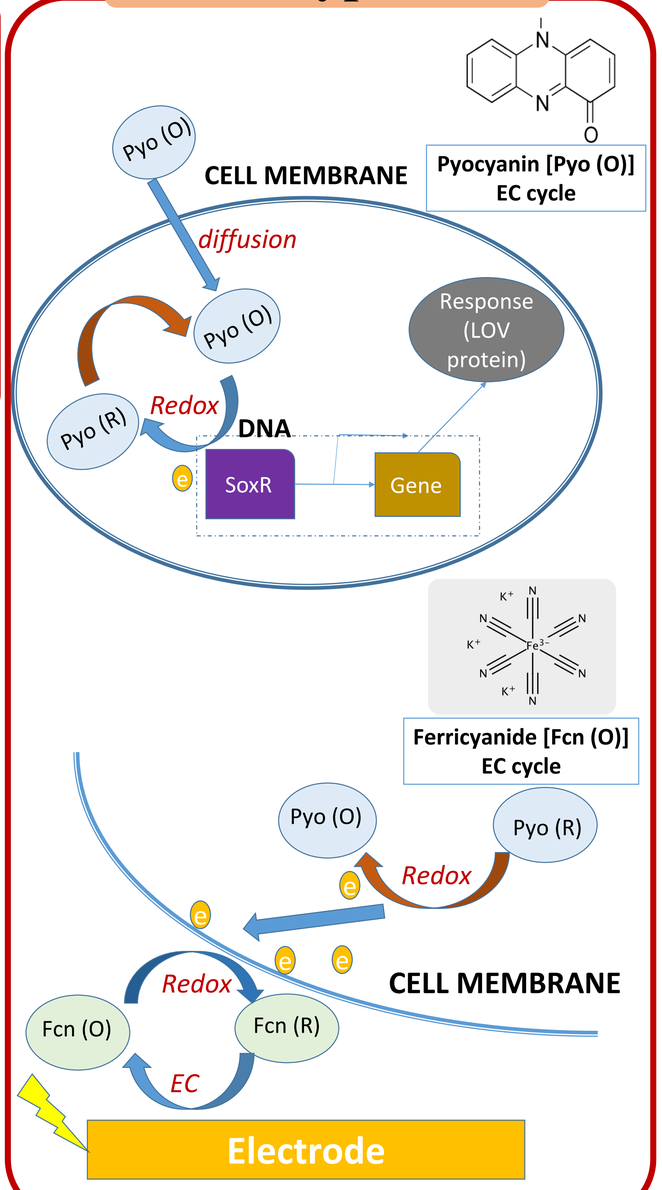
Introduction

Bioelectronic devices enable communication between biological and electronic systems. However, 'bioactuators' (devices that convert electronic signals to biological ques) are not commonly available as they require an electronic contact with the biological system to allow selective electronic communication transfer [1-3]. Here, we present a novel bioelectronic device comprises bi-modal electrochemical-optical lab-on-a-chip platform to study the behavioral response of single genetically modified bacterial cells. The lab on a chip is integrated with a microfluidic network chip to enable controlled and automated analysis of the cells' response to different chemical and electrical ques. Following electrochemical and flow validation of the lab on a chip, we demonstrate the successful electrical stimulation of the behavioral fluorescent response from the cells. We analyzed the stimulated fluorescent response, and we show the distribution among the cells (Figure 1). Surprisingly, we observe a different single-cell fluorescent response distribution between chemical and electrical stimulation ques.

Work Hypothesis



Methodology

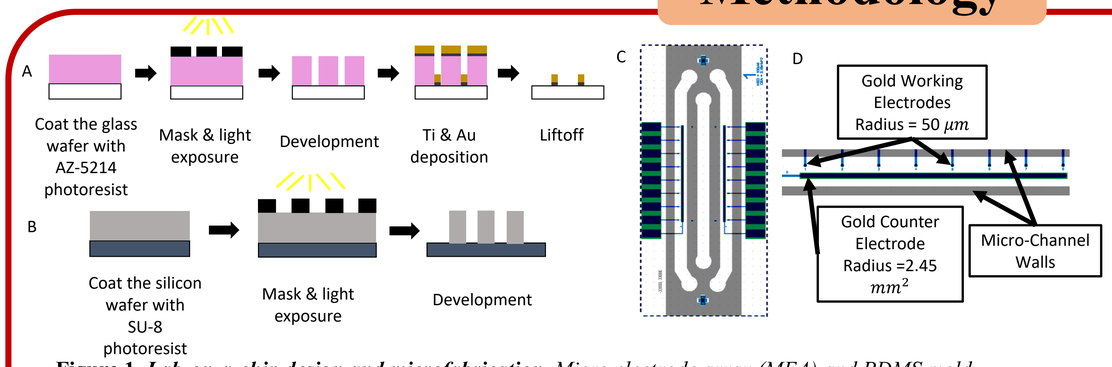


Figure 1. ***Lab-on-a-chip design and microfabrication.*** *Micro electrode array (MEA) and PDMS mold fabrication (A) MEA fabrication scheme (B) PDMS channel mold fabrication scheme (C) full flow electrochemical cell design (D) electrochemical cell design inside a specific channel*

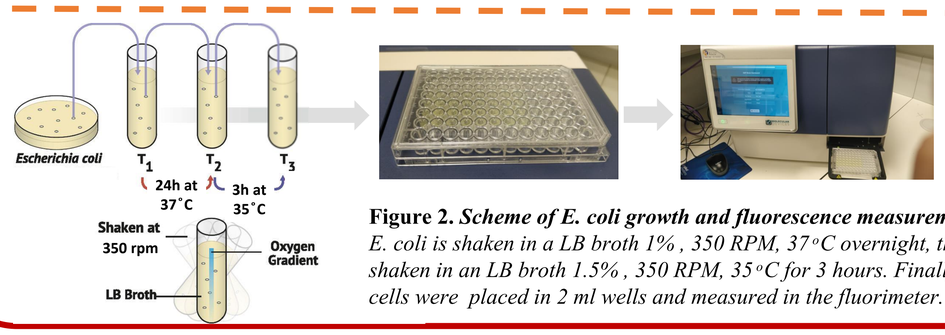


Figure 2. ***Scheme of E. coli growth and fluorescence measurement.*** *E. coli is shaken in a LB broth 1% , 350 RPM, 37°C overnight, then shaken in an LB broth 1.5% , 350 RPM, 35°C for 3 hours. Finally, the cells were placed in 2 ml wells and measured in the fluorimeter.*

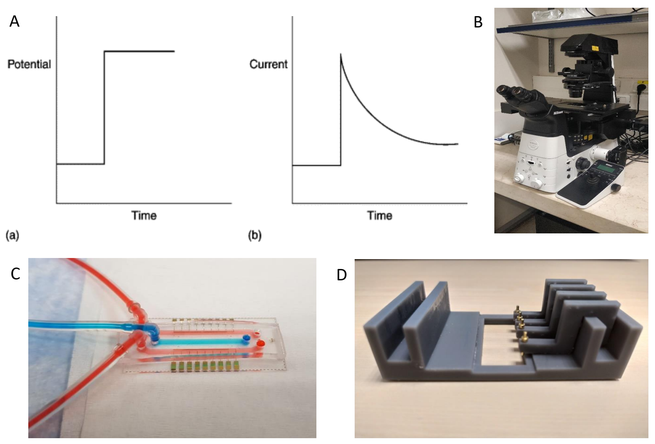


Figure 3. ***Electrochemistry, Optics, microfluidics and platform preparation*** *(A) Chronoamperogram example (B) Nikon Ti-2 Eclipse fluorescent microscope (C) flow test in the lab-on-a-chip with 3 independent channels (D) 3D printed platform for lab-on-a-chip electrical connections and placement under the microscope*

Results

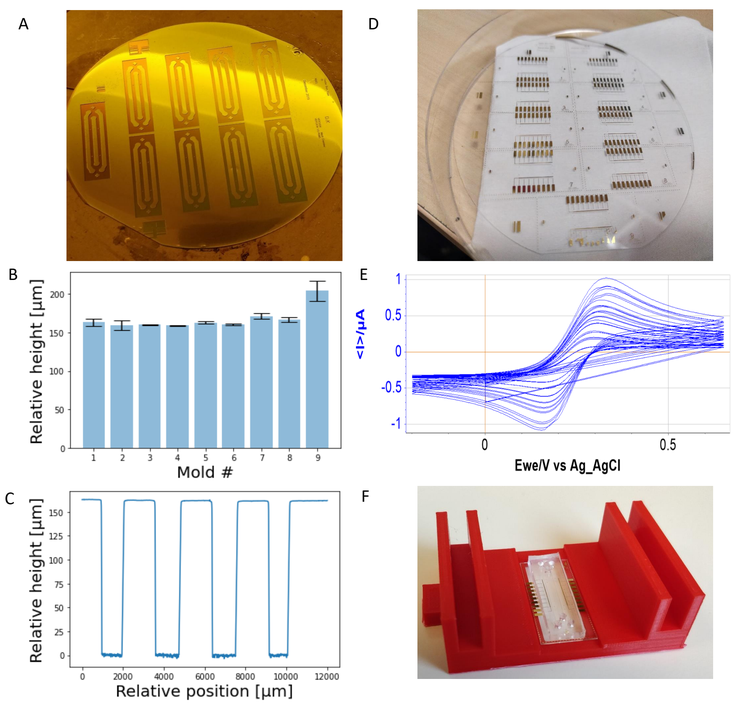


Figure 4. ***micro-electrode array and SU-8 mold fabrication and validation.*** *(A) fabricated SU-8 mold wafer (B) Relative height of each mold on the wafer (C) Relative height of specific mold (D) fabricated Au micro-electrode array wafer (E) cyclic voltammogram with various scan rates (10, 20, 50, 100, 150, 200, 300, 500, 800 mV/sec) (F) fabricated microfluidic lab-on-a-chip device placed on 3D printed platform*

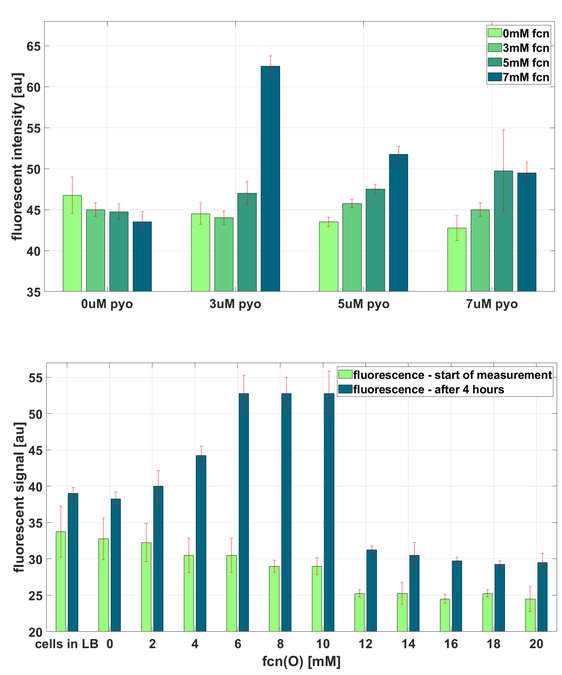


Figure 5. ***Bacterial cells were successfully activated in aerobic conditions.*** *Cells fluorescent response was optimized in the presence of ferricyanide and pyocyanin at aerobic conditions. Optimal conditions were chosen in the presence of 2 μm pyocyanin and 6-10 mM ferricyanide. (A) Fluorescent response of the cells in the presence of 0–7 μm pyocyanin and 0-7 mM ferricyanide. (B) Fluorescent intensity of the cells in the presence of 0-20 mM ferricyanide and 2 μm pyocyanin at the beginning (light green) and the end (dark blue) of the test. Tests were repeated in 8 wells containing 1 μL of E. coli bacteria (OD_{600} = 0.25).*

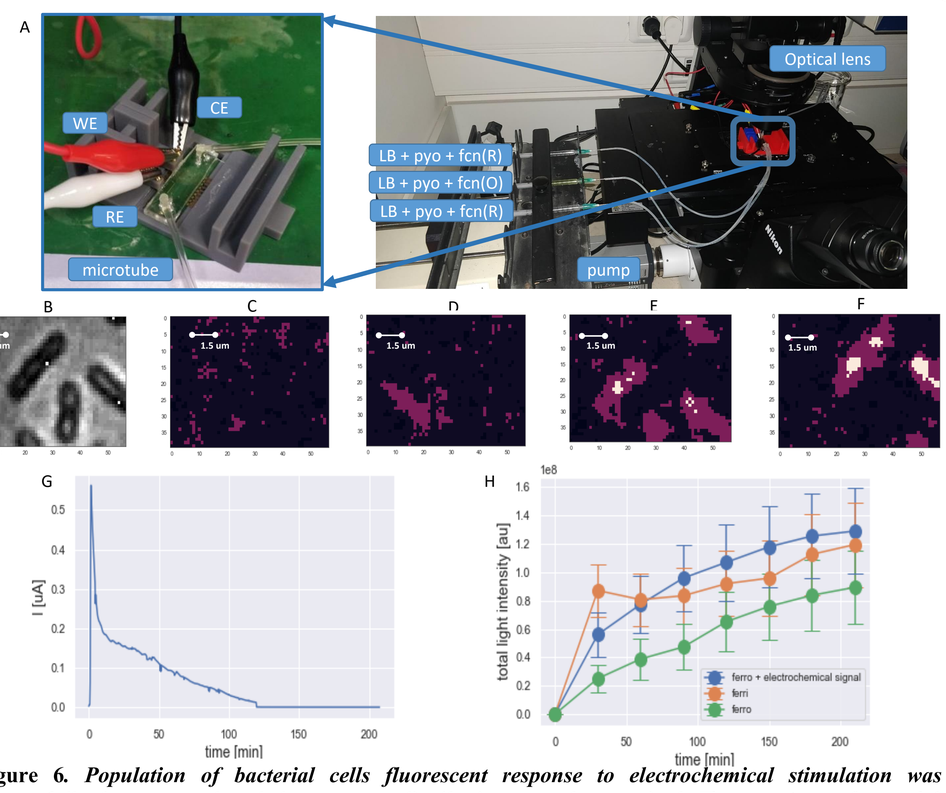


Figure 6. ***Population of bacterial cells fluorescent response to electrochemical stimulation was successfully demonstrated and the response distribution was characterized.*** *The experiment featured 3 independent groups of E. coli cells in different microchannels. All the groups contained cells in LB Broth (1%) and pyocyanin [2 μM]. Group 1 (blue) and group 3 (green) contained 8 mM ferrocyanide, while group 2 (orange) contained 8 mM ferricyanide. Group 1 was electrochemical stimulated. (A) experimental setup (B) Brightfield image of single bacterial cells. Fluorescent images of the single cells following electrochemical stimulation for (C) 0, (D) 1, (E) 2, and (F) 3 hours. (G) Chronoamperogram of the stimulated electrochemical signal at 0.5V. (H) Total fluorescent intensity measured from the microscope images of bacteria for different durations.*

Conclusions

1. We optimized the concentrations of Ferricyanide and Pyocyanin molecules to show the highest difference in fluorescent intensity.
2. We developed an integrated electrochemical and optical platform that enables visualizing single cells and allows activating and measuring fluorescent behavior of the cell.
3. Electrochemical stimulation of *E. coli* cells amplifies the fluorescent intensity on a single cell level. We observe significant difference in fluorescent signal between the three groups showing visual proof of electrochemical stimulation influence on fluorescent signal of the bacteria

Acknowledgements

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References

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