Probing Single-Bacterium Level Charge Transport in Microbial Fuel Cells

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Introduction: The capability of electrochemically active bacteria, such as *Shewanella* and *Geobacter*, to transfer electrons from metabolism of organic sources to electrodes without intervening catalysts serves as the basis for electricity production in microbial fuel cells (MFCs). MFCs have been the focus of increasing interest for renewable energy production because they feature long-term stability as self-sustaining systems, are able to operate at ambient or low temperatures with high-efficiency, and are tolerant of a broad spectrum of carbon feed stocks in wastewater through renewable biomass. While considerable progress has been made in improving MFC performance through the optimization of microbe selection and fuel cell design, the complex nature of biofilms in working MFCs has hindered a detailed understanding of charge transport at microbe/electrode and microbe/microbe interfaces. In this work, we report a "bottom-up" approach to investigate the extracellular electron transfer in MFCs at single- through multiple-bacterium level to elucidate the fundamental factors and limits determining power extraction.

Materials and Methods: We have developed a nanoelectrode platform where the interaction between individual bacterial cells and electrodes can be rationally controlled with a patterned array of nanoholes precluding or single window allowing for direct microbe-electrode contacts (Figure 1A). We designed the openings such that nanoholes and window exposed the same electrode area to solution so that the contribution from direct or mediated electron transfer (two limiting mechanisms that have been proposed) could be distinguished by comparing the current level from the two electrode configurations. The optically-transparent nanoelectrode chips were fabricated *via* lithographic methods; coupled with our specifically designed polydimethylsiloxane static/flow chambers and high-resolution phase-contrast microscopy, we were able to perform high-sensitivity electrochemical measurement while simultaneously resolving each cell on different electrodes.

Results and Discussion: We have carried out systematic electrochemical studies in two model systems, *Shewanella* MR-1 and *Geobacter* DL-1. Following the injection of *Shewanella* cells into the measurement chamber, short-circuit current recording showed similar changes for window and hole electrode, and was not correlated with the number of interacting cells, indicating that electron transfer occurs predominantly by mediated mechanism through the excretion of soluble redox molecules as "electron shuttles". For *Geobacter* cells, on the other hand, current generation was only observed on the window electrode, demonstrating the importance of *Geobacter*/electrode contact to initiate charge transfer. To better characterize the current output from single *Geobacter* cell, we improved the chip design to achieve more localized measurement in isolated, 40-μm deep wells. Quantized step-wise current increases of 92(±33) fA and 196(±20) fA were recorded (Figure 1B), and simultaneous cell tracking and current recording further revealed that the current steps were directly correlated with the contact of one or two cells with the electrodes (Figure 1C). Electrochemical characterizations after biofilm formation demonstrated the long-range charge transport capability of *Geobacter* cells through cell-to-cell electron hopping, which unfortunately became significantly diminished at the length scale >50 μm.

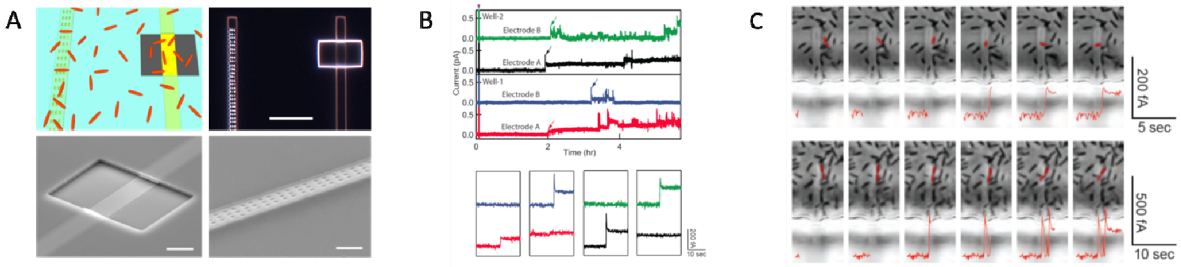

Figure 1 (A) Design and characterization of nanostructured electrodes for probing extracellular electron transfer. (B) The quantized current generation from individual *Geobacter* DL-1 cells. (C) Evolution of in-situ phase-contrast images of DL-1 cells on and around the measured electrode when the current spike occurs.

Conclusions: In this work, we unambiguously demonstrated the extracellular electron transfer mechanism in MFCs, and established the amount of current generated by an individual bacterium in the absence of a biofilm. These results highlight the potential upper limit of MFC performance, and the possibility to improve power extraction through facilitated long-range electron transfer, *e.g.*, by developing 3D, hierarchical bioanodes, or doping extracellular matrix with metal/semiconductor nanomateirals.