Improvement of Interdigitated Array Electrodes for the Investigation of Electron Transfer Through Biofilms of *Geobacter sulfurreducens* Mutants

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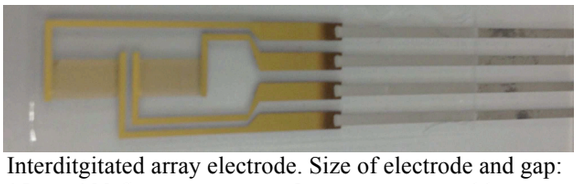
Geobacter sulfurreducens is an anaerobic bacterium capable of utilizing insoluble extracellular electron acceptors in respiration. In the laboratory, cells will quickly attach to electrodes poised at oxidizing potentials (~0.2 V vs. SHE), and use energy gained from acetate oxidation linked to electrode respiration to generate ATP and multiply. While many bacteria have been shown to be capable of this 'interfacial' electron transfer, *Geobacter* isolates are among the few that can continue to transfer electrons to surfaces even as cells stack upon each other. Within 3 days, biofilms thickness can be in excess of 20 µm, with cells at the top of the biofilm still respiring and utilizing the biofilm below for extracellular electron transfer to the electrode. New tools are needed to characterize the complete suite of proteins responsible for extracellular, between-cell electron transfer pathways, measure driving forces required, and understand how this natural conductivity can be harnessed.

Interdigitated array electrodes have been used to study rates of electron transfer within electrochemically active thin films since the pioneering work of Murray,[1] and were adapted by Heller for studies of electron diffusion coefficients within redox polymer hydrogels.[2] We have fabricated interdigitated array electrodes to allow measurements of electron transfer through wild-type *G. sulfurreducens* biofilms, in order to separate between-cell extracellular electron transfer events from respiratory events. A key goal of this work is to compare wild type biofilms to biofilms formed by mutants defective in key secretion pathways, extracellular cytochromes, or extracellular polysaccharide components. In order to perform multiple comparisons, the reliability, electrode design, and compatibility with bacterial cultivation had to be improved.

Interdigitated arrays were similar to those utilized in previous work,[3] containing two identical independently addressed arrays of one hundred 15 µm wide electrodes separated by 15 µm gaps. To mask printed gold busses and prevent them from serving as artifactual colonization sites, an aerosol printing technique depositing an optically clear UV-cured insulating layer was developed. Compared to previous masking approaches, printed electrodes were robust enough to allow repeated autoclave sterilization and electrochemical cleaning in strong acids. Additionally, the layout was altered (see image) to allow electrical connections outside of an anaerobic electrochemical cell, eliminating the use of solder and epoxies which often caused fouling of the surface and affected *Geobacter* attachment.



Interditgitated array electrode. Size of electrode and gap: 15µm. 100 fingers per array, 2 arrays per assembly.

These interdigitated arrays were used to grow biofilms of *G. sulfurreducens* wild type in electrochemical cells containing planar graphite or gold electrodes[4] to verify that rates of colonization, current production, and voltammetric characteristics were unaffected by the electrode design and repeatable within replicates. Use of multiple bipotentiostats allowed two strains to be grown in parallel, with two independent arrays in each electrochemical cell.

A key observation in previous work with interdigitated electrodes was that *G. sulfurreducens* biofilms were only conductive when the potential of source and drain electrodes spanned a narrow window centered at approximately -0.350 V vs. SHE, which is 80 mV lower than the midpoint potential of catalytic voltammetry. Work will be presented contrasting the conducted current profiles of mutants defective in inner membrane cytochromes (which are hypothesized to alter catalytic electron transfer) vs. mutants defective in extracellular cytochromes (which are hypothesized to alter between-cell electron transfer).

References

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3. R. M. Snider, S. M. Strycharz-Glaven, S. D. Tsoi, J. S. Erickson, and L. M. Tender, *Proceedings of the National Academy of Sciences*, **109**, 15467–15472 (2012).

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