DETECTING BACTERIAL TOXINS USING A NANOFLUIDIC ELECTRODE ASSEMBLY

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Introduction

Much interest in recent years has been placed on using electrode assemblies in microfluidic channels to perform real-time electrochemical measurements. However, the scale down of reference electrodes (REs) for microfluidic systems faces many challenges, such as the IR drop that occurs when REs are downstream or far away from the working electrode and dissolution of electrolyte from the RE.[1] The present work involves the use of an integrated miniaturized pseudo-reference electrode made from palladium (Pd) metal in a nanofluidic electrode assembly (NEA). Pd was chosen for its ability to absorb molecular hydrogen from the solution, allowing stable potential measurements without the need for special electrolytes, a desired trait in REs.[2] The Pd reference electrode (RE) was characterized against a standard Ag/AgCl RE in buffered conditions. The fabricated devices were then used to selectively detect production of pyocyanin by *Pseudomonas aeruginosa* (PA) in growth media. Pyocyanin is an electrochemically active toxin that is produced by the bacterium PA that helps it proliferate in human hosts.[3, 4] To date pyocyanin detection has only been accomplished using large scale electrodes

Materials and Methods

NEAs were constructed using standard microfabrication methods. Figure 1 shows a completed device and a schematic of the fabrication method. Optical lithography was used to create dozens of devices per run. The small dimensions of the nanochannel prevent cells from entering and interacting directly with the electrodes. Further, confining the electrodes inside a nanocavity, away from the bulk sample reservoir, decouples the measurements from external flow conditions.[5]

Electrochemical measurements were made with a bipotentiostat from CHI instruments. Solutions were prepared with ultra-purified water (resistance>18 Mohms). To characterize the constructed devices, ferrocene dimethanol dissolved in 1 M KCl at concentrations ranging from 1 to 1000 µM was used. Phosphate buffer (PB), as supporting electrolyte, was prepared as a 1 M stock at pH 6.8. 1 to 100 µM Pyocyanin solutions in PB were prepared for device testing.[6]

Results and Discussion

Using the fabricated NEA, pyocyanin was detected in solutions ranging from 1-100 µM in buffered solutions vs both the Pd RE and a commercially available Ag/AgCl RE. The resulting maximum currents for each pyocyanin concentration tested are shown in Figure 2. Three samples at each concentration were measured and a 0.597 µM detection limit for pyocyanin was obtained (Figure 2).

The NEA was also able to selectively detect pyocyanin produced by four different PA strains grown in trypticase soy broth at 37 °C for 8 days. Filtered samples were loaded directly onto the NEA without any pretreatment. Wildtype *PA14* produced the highest concentration of pyocyanin, followed by *pelA*, which cannot form dense biofilms, and *phzS* and *phzM* mutants, which cannot produce any pyocyanin (Figure 3).

Conclusions:

Pyocyanin detection using a microfabricated NEA with an integrated PD Re was accomplished. The ability to detect low concentrations of pyocyanin electrochemically opens the possibility for real-time studies of PA. Furthermore, the integrated NEA allows integration inside microfluidic systems to provide label-free chemical analysis of biologically relevant systems.

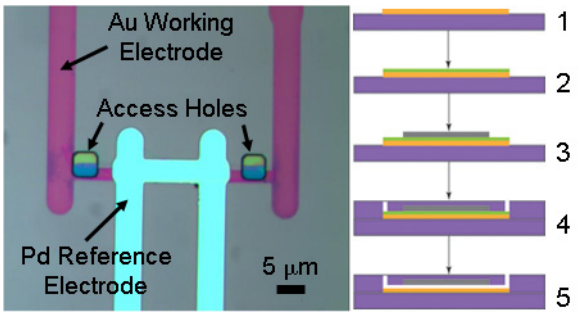

Figure 1: Left: Optical micrograph of NEA. Right: Fabrication Schematic. 1) 20 nm of Au patterned on SiO_2 wafer, followed by 2) 60 nm Cr, and 3) 120 nm Pd. 4) 550 nm of SiO_2 is deposited and access holes are created using RIE. 5) Cr sacrificial layer is removed with wet Cr etchant, yielding the finished device.

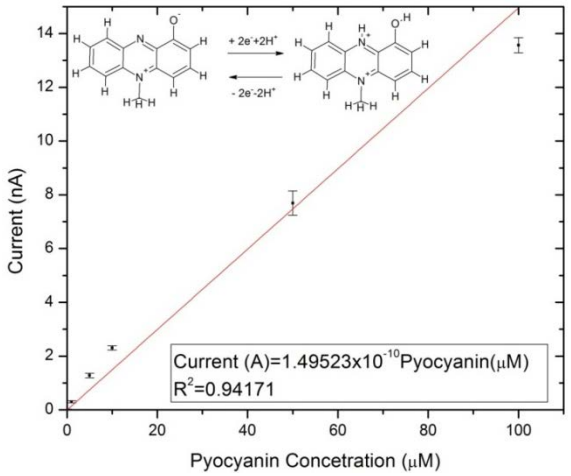

Figure 2: Plot of maximum current detected from solutions containing from 0 to 100 µM pyocyanin in 100 mM PB. The current from the blank has been subtracted out so that a solution containing no pyocyanin has a current of 0 A. Inset: The oxidized and reduced forms of pyocyanin.

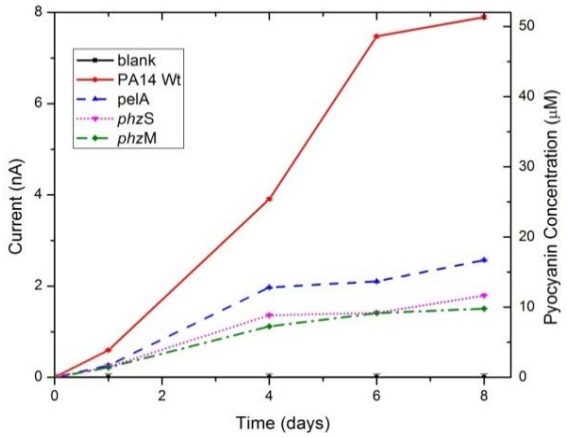

Figure 3: Pyocyanin production of P. aeruginosa strains PA14 wild type, pelA, phzS, phzM over 8 days of culture at 37 °C. pelA has a gene removed to prevent it from forming biofilms, phzS and phzM have had the genes for pyocyanin production removed, but can make precursor molecules

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