Electrochemically Detecting *P. aeruginosa* Toxins in Relevant Biomedical Samples

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Introduction

Pseudomonas aeruginosa is a prevalent opportunistic microbe that takes advantage of hosts with suppressed immune systems, such as wound victims and people with cystic fibrosis (CF).[1, 2] In fact, it has been shown that up to 80% of people suffering from CF will become infected with *P. aeruginosa* in their lifetimes.[3] *P. aeruginosa* continuously produces a blue-green pigment called pyocyanin that is unique to the species and produces detrimental effects to the host.[4, 5] Pyocyanin acts as a redox moderator and has been detected electrochemically in growth media.[6, 7] The present work looks at whether pyocyanin spiked into bronchial lavages, blood, sputum, and urine can also be electrochemically measured using disposable electrochemical cells, which would allow for faster identification of *P. aeruginosa* in these samples (<5 minutes).

Materials and Methods

Pooled blood, urine, sputum, and bronchial lavages from 20 healthy patients were purchased from bioreclamation.com. 500 µM pyocyanin stock was diluted into biological fluids at concentrations of 1-100 µM pyocyanin. Triplicate 150 µL samples were pipetted onto disposable carbon electrodes (Zensor) and scanned using square wave voltammetry (SWV) from -0.5 to 0 V at an amplitude voltage of 50 mV and 15 Hz. Measuring the oxidation peak of pyocyanin allows the limits of detection in these fluids to be established. All scans were performed vs. a commercial 1 M silver/silver chloride reference (CH Instruments Austin, Texas). To prove that the detection of pyocyanin electrochemically could be used as an indicator of *P. aeruginosa*, 150 µL of *P. aeruginosa* (PA14) cultures were inoculated in the above fluids ($\approx 2 \times 10^7$ cells/mL) and cultured for 1 day at 37˚C and then scanned.

Results and Discussion

Electrochemical scans of pyocyanin in blood, urine, sputum, and bronchial lavages yielded a linear relationship between pyocyanin concentration and the max current measured (Figure 1). Of interest were the different measured sensitivities for pyocyanin in each of the fluids (Slopes in Figure 2). This is attributed to the complexity of each fluid studied as well the differences in salt concentration and pH inherent to the samples tested. SWV scans of PA14 spiked into the above bio-fluids yielded a pyocyanin peak after one day of growth (Figure 3 shows response in urine).

Conclusions:

Pyocyanin was successfully detected in biological fluids spiked with pyocyanin or inoculated with *P. aeruginosa*. Of importance this is the only electrochemical molecule present in these fluids under the conditions used. The results indicate the potential to rapidly and selectively identify the presence of *P. aeruginosa* (via pyocyanin detection) in medically relevant samples.

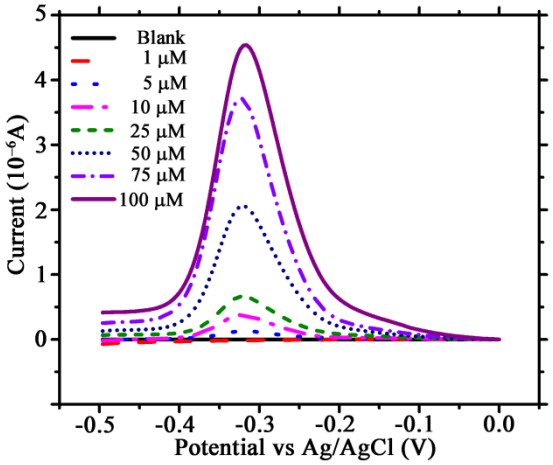

Figure 1: SWV scans of 1-100 µM pyocyanin in pooled bronchial lavages. Samples scanned at an amplitude voltage of 0.05 V and 15 Hz.

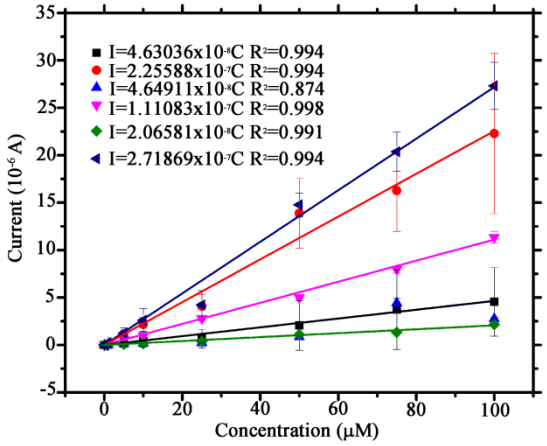

Figure 2: Maximum current versus pyocyanin concentration. Bronchial lavages (squares), urine (circles), blood no anticoagulant (upward facing triangles), sputum (downward facing triangles), blood with sodium heparin (diamonds), Lysogeny broth (sideways triangles). Equations for best fit lines for each biofluid: I = maximum peak current at a given concentration of pyocyanin (C).

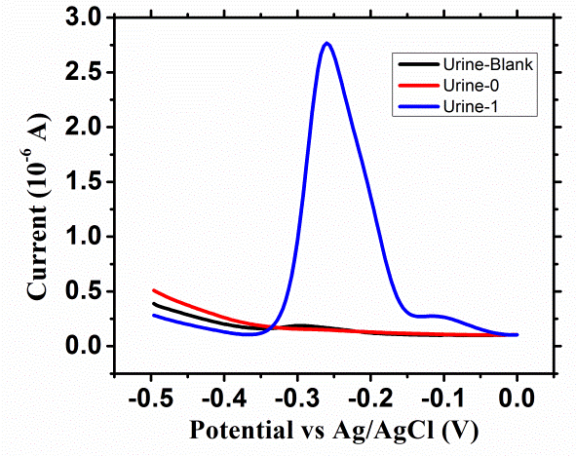

Figure 3: SWV of PA14 cells spiked into Urine. Scans were performed on blank biofluids, the fluids immediately after spiking with PA14 and 24 hours later.

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