Monitoring of Pseudomonas aeruginosa Toxins via Miniaturized Electrochemical Assemblies

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The production of pyocyanin from the opportunistic pathogen *Pseudomonas aeruginosa* was probed using a variety of miniaturized electrochemical systems. **Goal 1** used disposable screen printed carbon electrodes to measure pyocyanin in medically relevant samples showing for the first time that pyocyanin can be detected at medically relevant concentrations (1-100 µM) without sample processing. **Goal 2** coupled these same electrodes with Polydimethylsiloxane growth chambers to expose *P. aeruginosa* biofilms to varying concentrations of colistin sulphate. A reduction in electrochemical signal from pyocyanin, of approximately 80% at 100 mg/L colistin sulphate, highlights this molecules relation to biofilm health. **Goal 3** addresses the question of detecting *P. aeruginosa* in patient samples if the concentration of pyocyanin is initially low/non-existent in patient samples. Disposable screen printed carbon electrodes were embedded within King's A agar to determine whether electrochemical detection of pyocyanin could decrease the positive time to detection of *P. aeruginosa*. Measurements using bacterial loads of PA14 from 10^2-10^8 cells showed a load dependence on the electrochemical time to detection. Importantly the positive identification of *P. aeruginosa* was reduced approximately by 14-18 hours. **Goal 4** looks at the miniaturization of the reference electrode using palladium as a novel pseudo reference material. The reference electrode was fabricated in a nanofluidic chamber housing a gold working electrode. The palladium electrode showed promise as it maintained a stable reference during testing and was sensitive to changes in pH only. The miniaturized two electrode system was capable of discerning pyocyanin production from the wild type and three mutants of *P. aeruginosa*. **Goal 5** combines all of the necessary components for a three electrode electrochemical cell, within a nanofluidic channel. This was done to monitor the production of pyocyanin from small concentrations of *P. aeruginosa* confined in microfluidic channels to address whether pyocyanin production was indeed controlled by quorum sensing. Results suggest that the production and measurement of pyocyanin in this system is indeed dependent on quorum sensing. However the utilized system requires a 100 fold increase in the number of cells present to approach similar bacterial concentrations of other reported single cell quorum sensing platforms, indicating the results are inconclusive. Future research should be aimed at simplifying the fabrication and characterization processes of the three electrode nanofluidic sensors along with the miniaturization of the sensors. Decreasing sensor and growth chamber size will help to address the question of whether or not *P. aeruginosa* can successfully detect its own quorum sensing molecules leading to the production of pyocyanin.