Electrochemical Detection of *Pseudomonas aeruginosa* in Chronic Wounds

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Introduction

Here, we report the first use of electrochemical sensors to rapidly detect *Pseudomonas aeruginosa* in chronic wound patients to promote effective antibiotic stewardship and improved patient care by minimizing the delay between biological sample obtainment and its bacterial identification in the clinical setting. With the current use of plate cultures inoculated from swab samples taking anywhere from 24 to 48 hours before a positive result can be produced, there is an unmet need to develop rapid alternatives for bacterial identification. In clinical care, the highly resistant pathogen *P. aeruginosa* is one of the leading causes of bacterial infections among patients with cystic fibrosis, with compromised immune systems, and with chronic wounds.[1] A rapid diagnosis would allow a physician to switch from broad-spectrum antibiotics to more direct targeted therapy, thereby lowering antibiotic resistance and improving patient care outcomes.

To address this need, we report here the use of an inexpensive electrochemical sensor to detect pyocyanin, a unique, quorum-sensing molecule secreted by *P. aeruginosa*, in fluids obtained from patients with chronic wounds (Figure 1).[2] The redox-active pyocyanin molecule can be rapidly detected (under two minutes) using electrochemical sensors that require minimal sample preparation and only 7.5 microliters of sample to analyze.

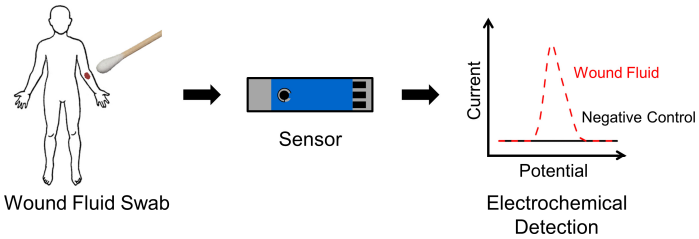

Figure 1. Detection scheme for pyocyanin production by *Pseudomonas aeruginosa* in clinical wound samples. A swab is taken from the wound and placed onto an electrochemical sensor. The presence (peak, red dashed line) or absence (no peak, black line) of pyocyanin indicates whether *P. aeruginosa* is present in the sample.

Materials and Methods

The screen-printed electrochemical sensor utilizes a carbon-based working (3 mm diameter disk) and counter electrodes, alongside a Ag/AgCl reference electrode. A total of 14 wound fluid

samples were tested. For each test, 7.5 µL of wound fluid was pipetted onto a sensor and square-wave voltammetry was used to determine the presence or absence of pyocyanin in the samples (Figure 2). For all of the experiments, square-wave voltammetric scans were performed at potentials ranging from -0.7 to 0.0 V at an amplitude voltage of 0.05 V, step voltage of 0.004 V, and a frequency of 15 Hz. The electrochemical results were compared against 16S rRNA sequencing to determine if *P. aeruginosa* was present in the sample.

Results and Discussion

Figure 2 shows an electrochemical square-wave voltammogram of wound fluid, where the observed current peak is due to pyocyanin oxidation, indicating the presence of *P. aeruginosa* in the sample. Comparing against 16S rRNA sequencing, the electrochemical results yielded 9 correct matches, 2 false negatives, and 3 false positives, giving a sensitivity of 71% and specificity of 57% for the detection of *P. aeruginosa*. The results indicate that this sensing platform may be a useful point-of-care test for this bacterium in human wound fluid. Additionally, despite the polymicrobial nature of human wound specimens, other redox-active molecules that would impede the sensor performance in a clinical setting were not observed. More importantly, the sensor can be functionalized to potentially detect other bacterial species by altering the applied potentials and thus can be enhanced into a point-of-care device with the capability to detect a wide range of clinically relevant bacterial pathogens.

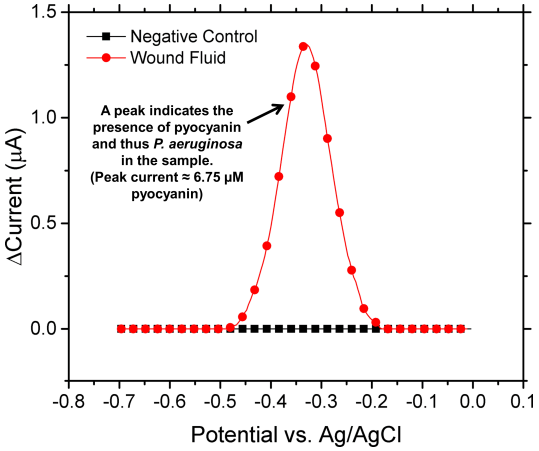

Figure 2. Square-wave voltammograms of clinical wound fluid and a negative control (growth media). The observed electrochemical peak is due to pyocyanin oxidation, indicating the presence of *P. aeruginosa* in the wound fluid. The concentration of pyocyanin (µM) in the sample can be calculated from the peak current (µA) using a calibration curve (not shown).

Acknowledgements

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References

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2. Sismaet, H.J., et al., *Electrochemical detection of Pseudomonas in wound exudate samples from patients with chronic wounds*. Wound Repair Regen, 2016, 24(2), 366-72.