

ELECTROCHEMICAL MONITORING OF *PSEUDOMONAS AERUGINOSA* BIOFILMS IN MICROFLUIDIC CHANNELS

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ABSTRACT

Polydimethylsiloxane microfluidic channels were irreversibly plasma bonded to microfabricated electrochemical sensors to monitor the presence of *Pseudomonas aeruginosa* biofilms. Channels were electrochemically monitored via square wave voltammetry to detect the presence of redox molecules produced by wild type strain PA14 biofilms growing inside the channels.

KEYWORDS: *Pseudomonas aeruginosa*, Biofilms, Electrochemical Detection

INTRODUCTION

Pseudomonas aeruginosa (PA) is an opportunistic pathogen responsible for many hospital acquired infections. It is especially problematic for patients suffering from compromised immune systems due to its ability to produce a variety of damaging virulence factors [1-4]. These virulence factors allow PA to establish biofilms within infected hosts prolonging infections. Of these virulence factors the phenazine pyocyanin is of interest because it can be measured electrochemically [5, 6]. The ability to electrically monitor the production of pyocyanin could give insight into the health of the growing biofilms, and may be useful as a measure of antibiotic efficacy. This paper describes the microfabrication of a microfluidic channel containing an electrochemical sensor to monitor PA biofilms via the production of pyocyanin.

The electrochemical monitoring of bacterial biofilms is not new. Previous researchers have shown that PA biofilms can be monitored via electrochemical impedance spectroscopy (EIS) [7] and cyclic voltammetry (CV) [8]. While effective at monitoring the growth of cells on the electrode surface through impedance changes, EIS is unable to recognize the production of electrochemical molecules. Cyclic voltammetry is a well-known electrochemical technique capable of monitoring redox reactions. However, it suffers from large charging currents lowering its sensitivity. In this work, the authors opted to use square wave voltammetry (SWV) due to its increased sensitivity and its ability to measure redox reactions compared to CV and EIS respectively. Previous research has shown that pyocyanin production can be monitored by SWV using microfabricated sensors [9, 10], but this is first time it has been used to monitor biofilms of PA within a microfluidic channel.

EXPERIMENTAL

Microfabricated interdigitated electrode arrays were patterned on a glass wafer with a single photolithography mask, followed by electron beam deposition and metal lift-off. The electrode arrays consisted of a pair of gold interdigitated electrodes, as well as a gold counter and quasi-reference electrodes covered in a passivating layer of SiO₂. Access holes to the electrodes were etched with SF₆ through the SiO₂ using ion coupled plasma. Polydimethylsiloxane (PDMS) microfluidic channels were created using replica molding of a silicon master wafer patterned with AZ4620 resist. The glass substrate containing electrode arrays was diced, and PDMS channels were aligned and plasma bonded over the arrays. A finished device can be seen in Figure 2.

Overnight cultures of wild type strain PA14 were used for all biofilm measurements. Cells were centrifuged at 10,000 rpm for three minutes and the resulting pellet was re-suspended in trypticase soy broth (TSB). Completed devices contained two parallel PDMS channels (100 μ m wide) with microfabricated electrodes. One channel was filled with PA14 in TSB while the other was filled with only TSB (control). SWV was used for all electrochemical measurements.

RESULTS AND DISCUSSION

PA14 cells reconstituted in fresh TSB were loaded into the microfluidic channel. Cells were then allowed to grow under stagnant conditions. SWV scans were taken at set time intervals. As PA cell populations grow pyocyanin should be produced. The production of pyocyanin will result in an oxidation peak in the voltage range used (Figure 1). In this window it has been shown that the major electrochemical product is pyocyanin [5].

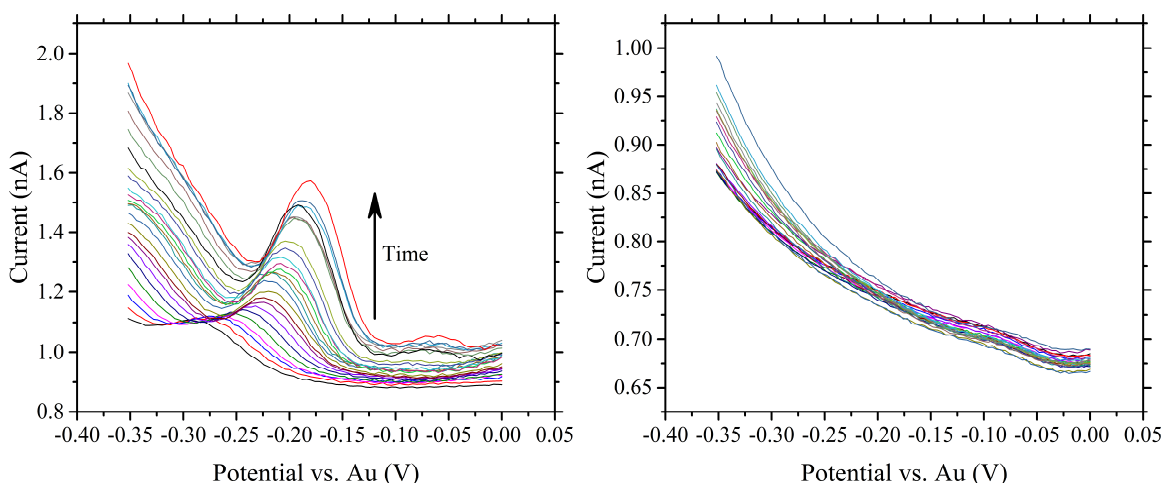


Figure 1: Left- SWV of left channel containing PA14. Right- SWV of right channel containing no bacteria. SWV taken at frequency and amplitude voltage of 15 Hz and 50 mV, respectively, from -0.35 to 0 V.

Figure 1 highlights the ability of the fabricated system to successfully detect the presence and absence of produced pyocyanin over time. The absence of any electrochemical or optical change in the channel containing only TSB makes it an effective control for the positive identification of PA14 and indicates that the fabricated system prevented cross contamination between channels (Figure 2). Previous research has shown that in the voltage window used, PA is the only clinically relevant bacteria that produces any detectable oxidation peaks [11], potentially enabling this system to detect the presence of PA in co-cultures as would be found in patient samples. After baseline subtraction the produced electrochemical response due to pyocyanin was more pronounced (Figure 2), simplifying the identification of the PA biofilm. While pyocyanin can be measured optically, since it is a blue pigment,

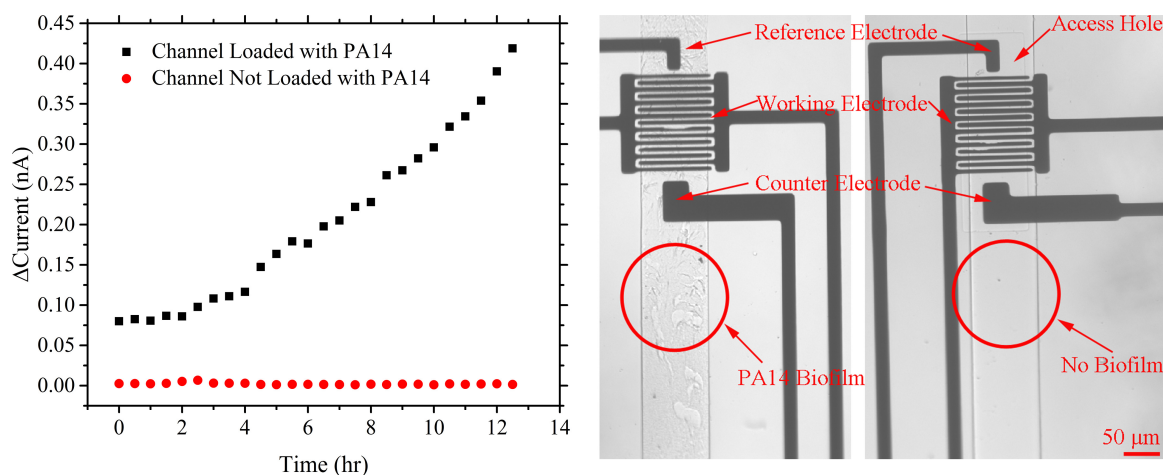


Figure 2: Left- peak currents, after baseline subtraction, for channel filled with PA14 (black squares) and control channel (red circles). Right- Optical comparison of a channel with PA14 growing in it vs. one with no bacteria present.

it would be very difficult to achieve this in our system due to the small path length, unless a considerable concentration of pyocyanin was being produced.

CONCLUSION

A microfabricated electrochemical sensor utilizing a microfluidic chamber has shown its ability to monitor PA biofilms via the production of pyocyanin. The system was able to differentiate the electrochemical response of a blank containing no PA compared to a PA biofilm. Due to the confined volumes in the microfluidic chamber this system could potentially be used to electrochemically screen novel anti-biotics' efficacy against PA biofilms via the production of pyocyanin.

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