Real-time In-situ Monitoring of Air Interface Pseudomonas aeruginosa Biofilms Growth and its Antibiotic Susceptibility Using a Novel Dual-chamber Microfluidic device

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BACKGROUND

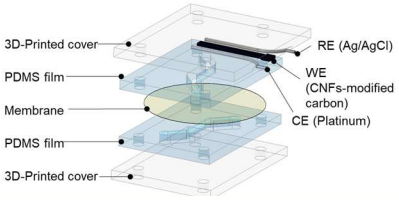
- Biofilms are communities of microorganisms, embedded in a self-produced matrix of extracellular polymeric substances (EPS) [1].
- Many chronic lung infections are caused by persistent *Pseudomonas aeruginosa* biofilms that developed in the respiratory tract [2].
- Current biofilm studies are focused on mono-interfaces and have neglected more realistic approaches, where diverse interfaces are involved.
- Conventional biofilm characterization tools are also usually destructive, time consuming and costly.
- The electrochemical technique offers an economical and effective solution to analyse biofilm viability in real-time, in-situ and in a non-invasive manner.
- *P. aeruginosa* produces an electro-active molecule, pyocyanin (PYO), which can be a useful marker of cell viability and virulence [3].

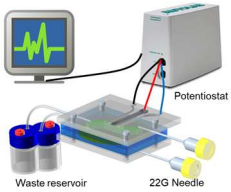
AIMS & OBJECTIVES

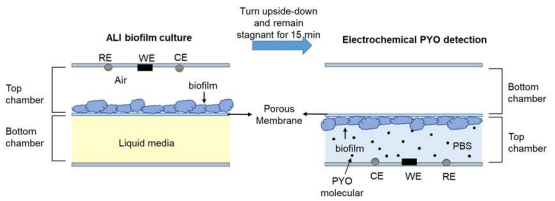
- Develop a novel electrode-integrated microfluidic device for culturing biofilm at the air-liquid interface (ALI) and perform real-time in-situ electrochemical biofilm detection.
- Investigate the growth characteristics of *P. aeruginosa* biofilm within the novel electrode-integrated microfluidic device.
- Evaluate the biofilm-killing efficacy of ciprofloxacin (CIP) antibiotic solution exposed to the apical surface and the basolateral substratum of the biofilm.

METHODS

- Three electrodes were integrated into a dual-chamber microfluidic design.
- The platform was then used for biofilm culture at the ALI and electrochemical detection.
- Electrochemical detection of the PYO produced by the biofilm grown in the device over time was performed.







- Square wave voltammetry (SWV) was performed using a potentiostat to quantify the concentration of PYO produced by the biofilm.
- Antibiotic solutions of different concentrations (0-1600 µg/mL) were delivered to the apical or the basolateral chamber of the microfluidic platform to mimic targeted respiratory delivery and systemic drug administration, respectively.

RESULTS

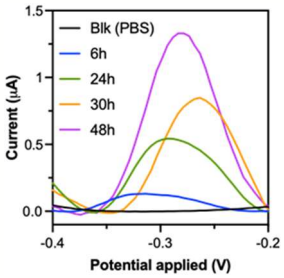


Figure 1. SWV scans of pyocyanin produced by ALI *P. aeruginosa*.

- Peak currents was observed at all time points (**Fig.1**) demonstrating that the *P. aeruginosa* bacteria cells cultured at the ALI are constantly producing PYO.
- The amount of PYO produced by the ALI biofilm correlated to its viable cell numbers (**Fig.2**).
- The PYO production rate and bacteria proliferation rate vary over time, with peak PYO production was observed between 24 h to 30 h (**Table 1**).
- PYO production reduced significantly after CIP solution treatment solution (**Fig. 3(a)**).
- Direct exposure of CIP on biofilm surface shows better biofilm-killing outcomes (**Fig.3 (b)**).

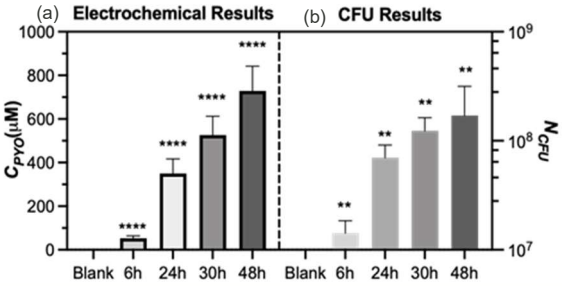


Figure 2. (a) The accumulated production of PYO detected by the electrochemical method. (b) The CFU number of biofilm samples. The significant difference compared to the blank control group (unpaired t-test) as indicated (N=6, mean ± SEM; **, P<0.01; ****, P<0.0001).

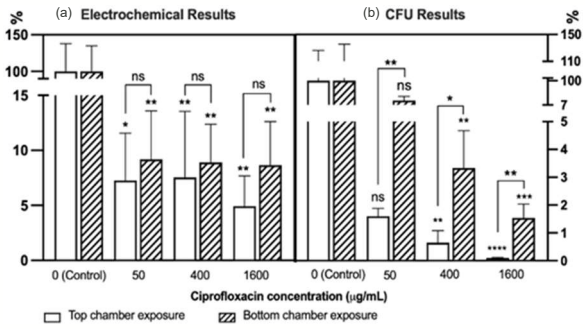


Figure 3. Comparison of biofilm eradication effect of CIP (50, 400, and 1600 µg/mL) for 6h exposure on 48h-old biofilms cultured on ALI. * over error bar indicates statistical significance obtained by the ANOVA test results comparing the treatment groups to the control group. * over the line indicates the statistical significance obtained by the unpaired tests comparing the data between two different drug delivery approaches using the same CIP concentration. (N=6, mean ± SEM; *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001; ****, p-value<0.0001; ns, no statistical significance).

Table 1. Averaged biofilm bacteria proliferation rate and PYO production rate over time.

Growth period	Bacteria proliferation rate (CFU/h)	PYO production rate (µM/h)
Attachment ~ 6h	3.2×10^6	8.85
6 h ~ 24 h	3.0×10^6	16.52
24 h ~ 30 h	8.9×10^6	29.27
30 h ~ 48 h	2.6×10^6	11.24

CONCLUSION S & FUTURE WORK

- The study successfully developed a novel electrode-integrated microfluidic device that for culturing biofilms at the ALI and performing real-time and in-situ electrochemical biofilm detection and quantification.
- The growth characteristics of *P. aeruginosa* biofilm developed at the ALI were measured in real-time and in-situ.
- The biofilm-killing efficacy of CIP antibiotic solution was found to be more effective when biofilms were exposed to the apical surface compared to the basolateral substratum of the biofilm.

References:
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[2] Maurice, N. M., Bedi, B. and Sadikot, R. T. Pseudomonas aeruginosa Biofilms: Host Response and Clinical Implications in Lung Infections. American Journal of Respiratory Cell and Molecular Biology, 58, 4 (2018), 428-439.
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