Development of a Microfluidic-Based Electrochemical Cell for Analyzing Bacterial Biofilms

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Abstract

Introduction: Biofilms are surface adhered bacteria secured inside of a matrix of extracellular polymeric substance(EPS) which can cause biofouling, biocorrosion[2], and persistent infections[3] while being beneficial in other areas. Their ubiquitous nature has led to a growing need and desire to detect, control, and maintain or remove them. The mechanical properties of the EPS matrix are the primary factor in determining biofilm stability and viability. These properties have been shown to be dependent on both stage of development[1] and environmental conditions[4,5]. Therefore a robust testing platform that allows for multiple analytical techniques to be performed is required. We have developed a novel self-contained microfluidic based electrochemical cell, shown in Figure 1, which utilizes electrical impedance spectroscopy (EIS) to measure the complex impedance of the biofilm as a function of the development stage while allowing for external confocal laser scanning microscopy and end of experiment atomic force microscopy techniques document their properties. The integration of the EIS measurements into the flow cell requires precise control of the region where bacteria colonize the surface. This guarantees that the reference and counter electrodes remain clean throughout the measurement time frame to provide accurate results.
Use of the COMSOL Multiphysics® software: The microfluidic cell, which operates in the laminar flow regime, was developed utilizing the Laminar Flow and Transport of Diluted Species physics of the COMSOL® software. Pseudomonas aeruginosa bacteria, a bacterium known to generate biofilms, were modeled as a dilute chemical species with a specified diffusion rate[6]. In all studies the bacteria were introduced through Inlet 2 at a concentration of 1[mol/m3] in growth medium while sterile medium is introduced through Inlets 1&3. All outlets were defined as pressure conditions with 0[Pa] and suppressed backflow. Multiple parametric studies were performed with these conditions in order to analyze the effect of varying the flow cell geometry, shown in Figure 2, and the inlet flow rates (results not shown) on the resulting bacterial diffusion profile.
Results: The results from the Multiple Inlet-Outlet model, Figure 2-A, are shown in Figure 3. From these results it can be seen that only changes to the Central Outlet Fan Width condition have a non-negligible effect on the resulting bacteria diffusion profile. The results from the Nozzle Geometry model, Figure 2-B, are shown in Figure 4. From these results it can be seen that all the Nozzle Geometry configurations perform better than the previous geometric configurations in containing the range of bacterial diffusion.
Conclusion: The results of this study show that it is possible to miniaturize an electrochemical cell with controlled bacterial growth for biofilm analysis, allowing precise studies of colonization and the early stages of growth with dramatically reduced amounts

of reactants and generated waste.

Reference

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Figures used in the abstract

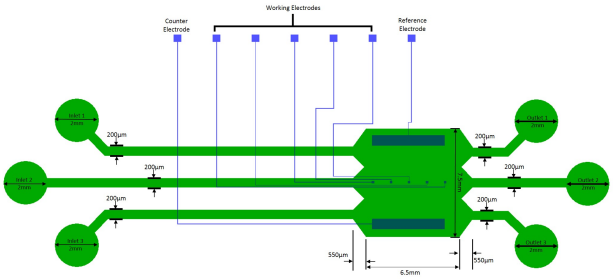
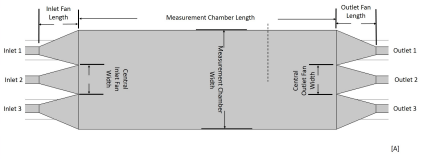


Figure 1: Figure 1 Initial Device Design. Microfluidic portions of the device are shown in green. Electrochemical portions of the device are shown in blue. Dimensions of the device are shown in black.

Dimension	Multiple Inlet-Outlet Model Parameters
Measurement Chamber Length	6.5[mm]*
Measurement Chamber Width	7.5[mm]*
Flow Cell Depth	100[µm]*
Inlet Width	600[µm]*
Outlet Width	600[µm]*
Inlet Flow Rates	3.48[µl/min]*
Diffusion Rate	10e-6 [cm²/s]*
Inlet Fan Length	100[µm], 550[µm]*, 1000[µm]
Central Inlet Fan Width	400 [µm], 2200[µm]*, 4000 [µm]
Outlet Fan Length	100[µm], 550[µm]*, 1000[µm]
Central Outlet Fan Width	400 [µm], 2200[µm]*, 4000 [µm]



Dimension	Nozzle Geometry Model Parameters
Measurement Chamber Length	6.5[mm]
Measurement Chamber Width	7.5[mm]
Flow Cell Depth	100[µm]
Inlet Width	600[µm]
Central Outlet Width	600[µm], 1000[µm], 1400[µm]
Inlet Flow Rates	3.48[µl/min]
Diffusion Rate	10e-6 [cm²/s]
Inlet Fan Length	550[µm]
Central Inlet Fan Width	2200[µm]
Outlet Fan Length	550[µm]
Central Outlet Fan Width	2200[µm]

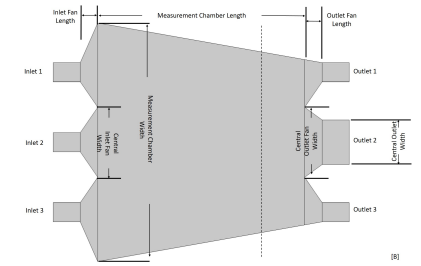


Figure 2: Figure 2 Geometry layout for COMSOL simulations. [A] shows the geometry of the Multiple Inlet-Outlet model that was parametrically studied. [B] shows the geometry of the Nozzle Geometry model that was also parametrically studied. The parameters that were varied are shown in the respective tables. The dashed lines on both figure show the location of future analysis. Values marked with an * represent baseline used as comparison for the Nozzle Geometry studies.

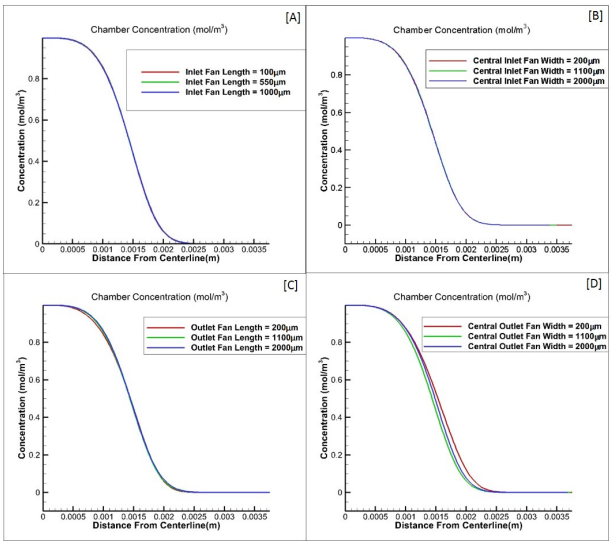


Figure 3: Figure 3 Concentration profile results for the Multiple Inlet-Outlet model. The concentration of bacteria at the line marked in Figure 2-[A] for one half of the measurement chamber is shown with respect to the centerline of the chamber. The variation for each case is as follows: [A] Inlet Fan Length [B] Inlet Fan Width [C] Outlet Fan Length [D] Outlet Fan Width.

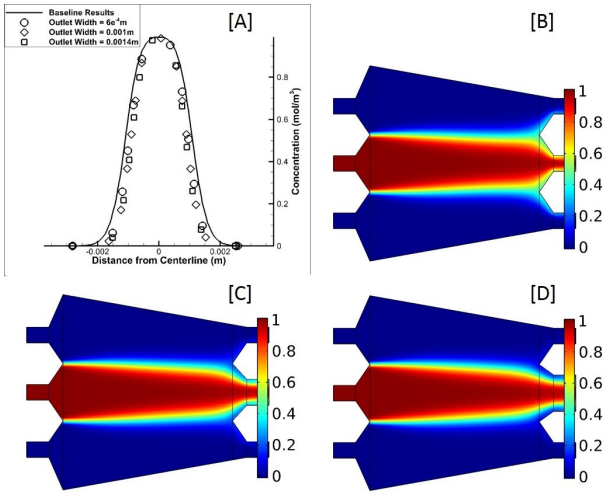


Figure 4: Figure 4 Nozzle Geometry model results. [A] Bacteria concentration profile for Nozzle geometry cases and for the baseline measurement chamber as discussed in Figure 2. [B] Concentration profile for 600µm wide central outlet. [C] Concentration profile for 1000µm wide central outlet. [D] Concentration profile for 1400µm wide central outlet. The color bars show concentrations in mol/m^3