Optimizing Bacterial Adhesion to a Microfluidic Platform for Monitoring Bacterial Biofilm Growth

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INTRODUCTION

Objective: Optimize bacterial adhesion in a microfluidic device using poly-L-lysine and fibrinogen

- Bacteria undergo cell-cell communication through uptake and release of autoinducers (signaling molecules)
- Reaching population threshold, a change in gene regulation leads to biofilm formation

Bacterial Biofilms:

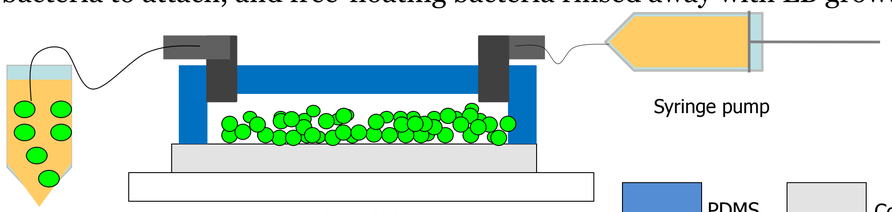
- Composed primarily of bacteria, polysaccharides, and proteins
- Prevalent in microbial infections of the body, with increased antibiotic resistance
- Bacterial adhesion is the first stage in biofilm development

Studying Biofilms:

- Biofilms can be optically monitored in microfluidics, with the advantages of a highly controllable environment, amplified sensitivity, inexpensive setup, and parallel operation
- Poly-L-lysine (polypeptide of L-lysine) and fibrinogen (plasma protein) shown to increase bacterial adhesion in generic bacteria, may increase repeatability of biofilm formation in microfluidics

MICROFLUIDIC PLATFORM

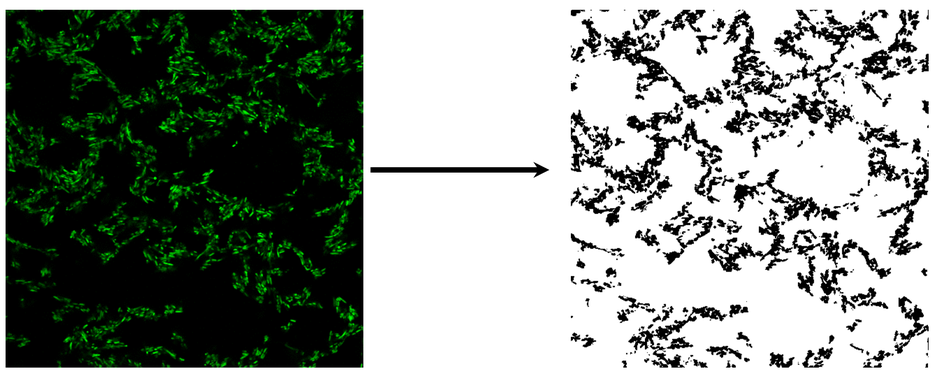
- A biocompatible transparent polymer (PDMS) used to create microfluidic channel
- Fluidic tubing connected to syringe and ports for interfacing the channel
- Design enables up to 6 lab-on-a-chip devices to be operated in parallel
- Using syringe/tubing, device is sterilized, inoculated with bacteria, incubated to allow bacteria to attach, and free-floating bacteria rinsed away with LB growth media



Cross-section of platform

DATA COLLECTION

- Bacteria in channel imaged using fluorescence microscopy
- Images analyzed using ImageJ to evaluate bacterial adhesion

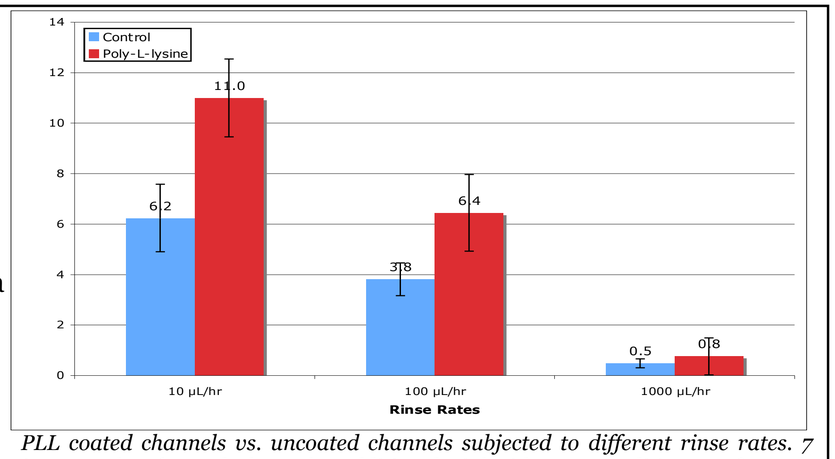


Fluorescence image *ImageJ analysis*

RESULTS

Poly-L-lysine Testing:

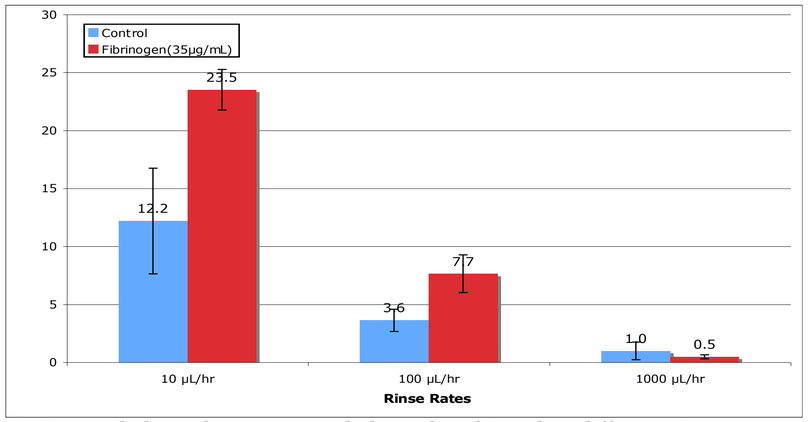
- 0.01% poly-L-lysine flowed into channel, dried overnight
- Channel inoculated with bacteria of OD_{600} 1.2, incubated 2hrs at 37°C, rinsed with LB growth media 30min
- 2 additional experiments testing only rinsing at 10 µL/hr yielded similar results



PLL coated channels vs. uncoated channels subjected to different rinse rates. 7 images taken for each channel. 1 additional trial with same conditions performed

Fibrinogen Testing:

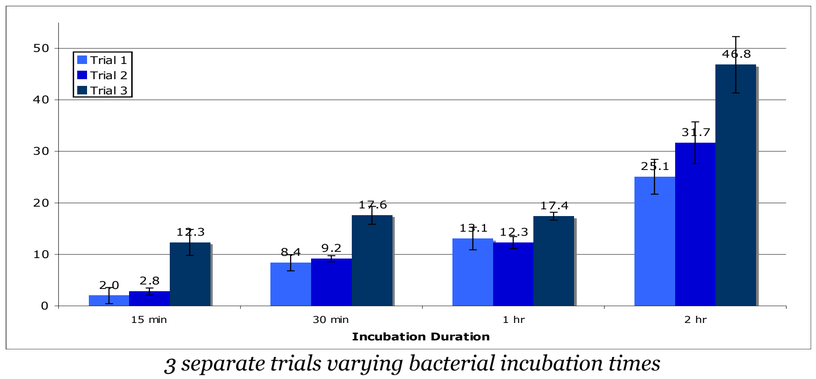
- 35 µg/mL fibrinogen in PBS flowed into channel, incubated for 1hr at 37°C, rinsed 30min at 50 µL/hr with PBS
- Bacteria of OD_{600} 1.2 inoculated channel, incubated 2hrs at 37°C, rinsed with LB growth media 30min.
- 5 additional experiments testing only rinsing at 10 µL/hr yielded similar results



FG coated channels vs. uncoated channels subjected to different rinse rates. 7 images taken for each channel. 2 addition trials with same conditions performed

Incubation Testing:

- Varying bacterial incubation times with uncoated channels
- Bacteria of OD_{600} 1.3 inoculated channel, incubated at 37°C, rinsed with LB growth media 30min at 10µL/hr



3 separate trials varying bacterial incubation times

CONCLUSION

- Poly-L-lysine and fibrinogen shown to improve bacterial adhesion to the microfluidic device
- Bacterial adhesion decreases as flow rate of rinsing procedure increases
- Longer the incubation time, the more bacteria attaches to surface
- Incorporation of the results will improve biofilm repeatability in the microfluidic device

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