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

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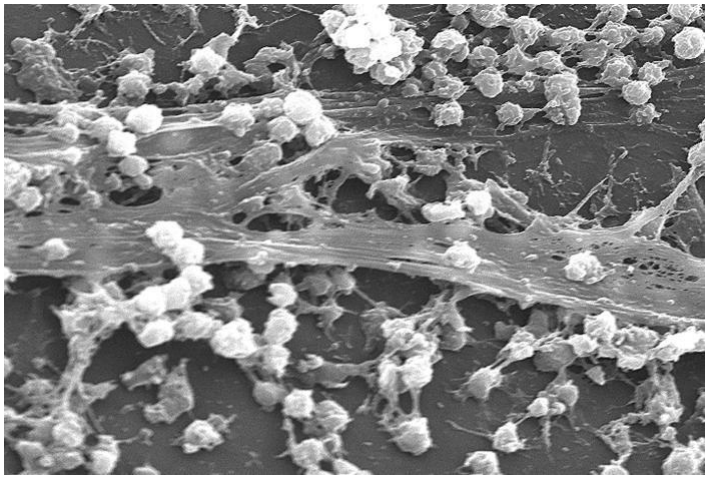
Bacterial Quorum Sensing

- Quorum Sensing
  - Cell-cell communication mechanism for unicellular organisms
  - Uptake/release of chemical signaling molecules
  - 3 principal classes of signaling molecules:
    - *E coli.* : autoinducer-2 (AI-2) - “universal” between Gram-positive and Gram-negative
- Changes in gene expression if bacterial population threshold reached
  - Ex. production of biofilm matrix, toxins

Bacterial Biofilms

- Biofilms:
  - Composed primarily of polysaccharides, protein, DNA, and bacterial cells
  - Prevalent in infections (respiratory tract, urinary tract) and foreign implants (heart valves, joint prostheses)
  - Dense matrix - increased antibiotic resistance



Biofilm on inserted catheter, www.cdc.gov

QuickTime™ and a
decompressor
are needed to see this picture.

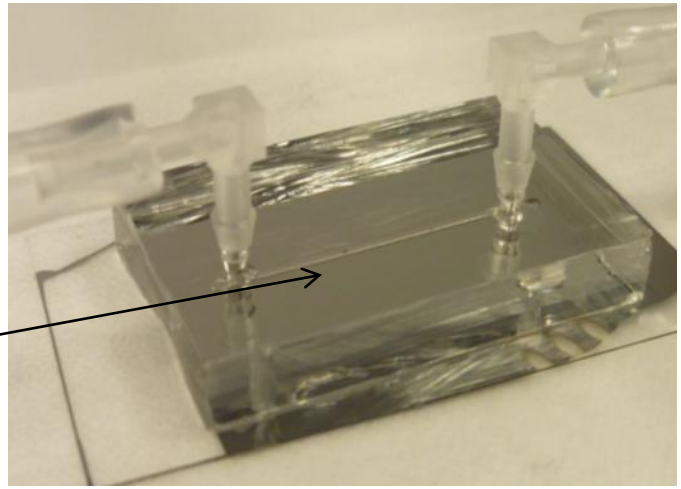
3D image of bacteria adhesion (confocal microscopy)

Studying Biofilms

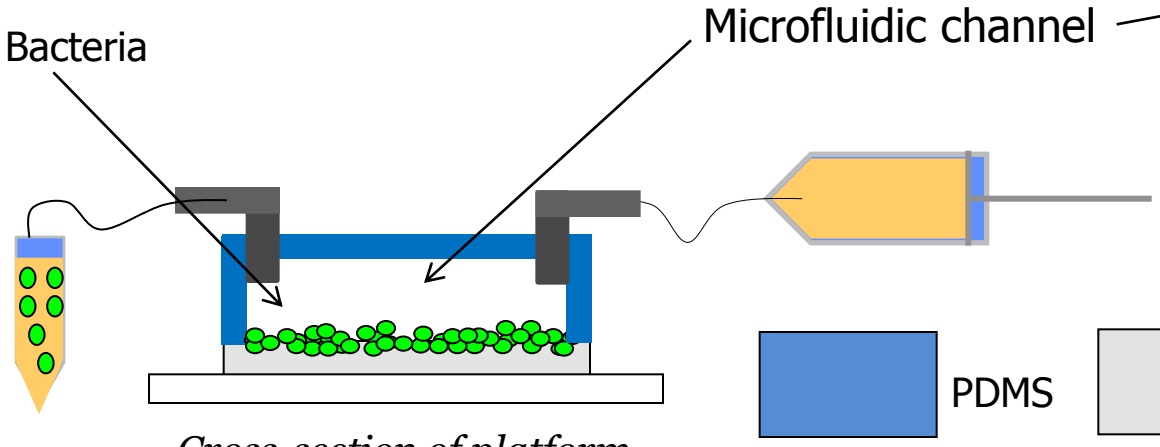
- Real-time optical monitoring of biofilm growth in microfluidics
- Advantages:
  - Highly controllable environment
  - Inexpensive, fast setup
  - Parallel operation
- Poly-L-lysine and fibrinogen shown to increase bacterial adhesion in generic bacteria - may increase repeatability of biofilm formation

Microfluidic Platform

- A biocompatible transparent polymer (PDMS) used to create microfluidic channel
- Device sterilized, inoculated with bacteria, rinsed with LB growth media



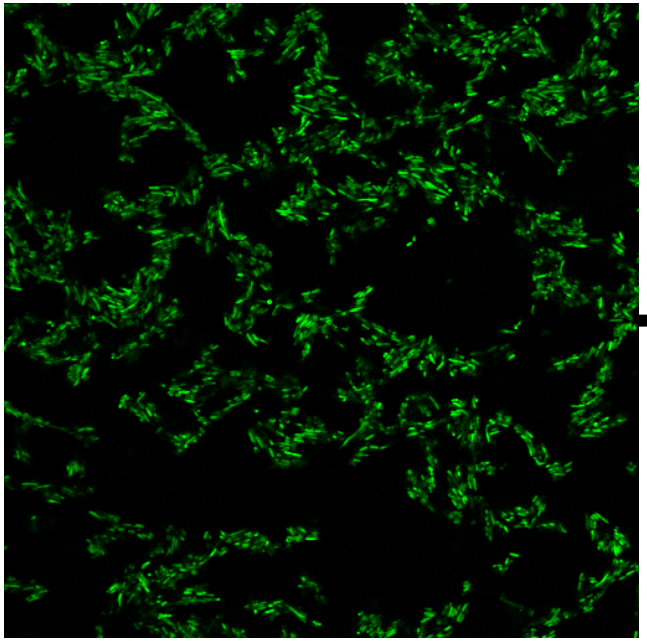
Microfluidic device



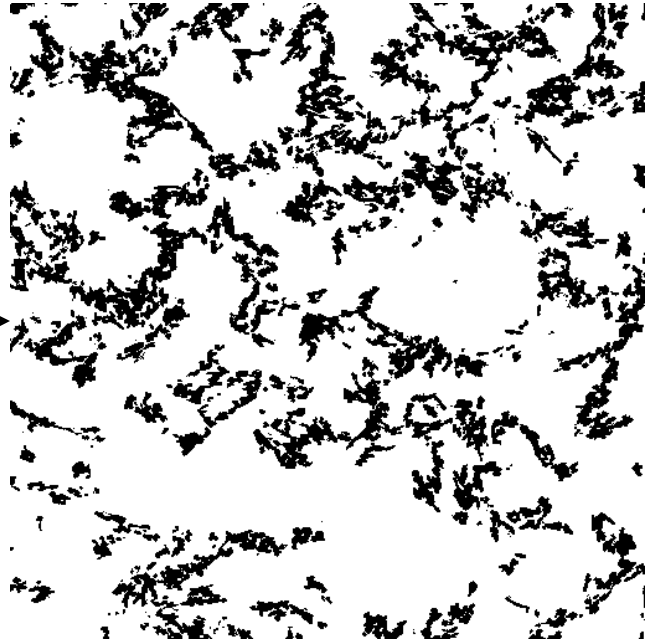
Cross-section of platform

Observing and Evaluting Adhesion

- Image bacteria in the channel using fluorescence microscopy
- Images analyzed using ImageJ to calculate percent area covered by bacteria



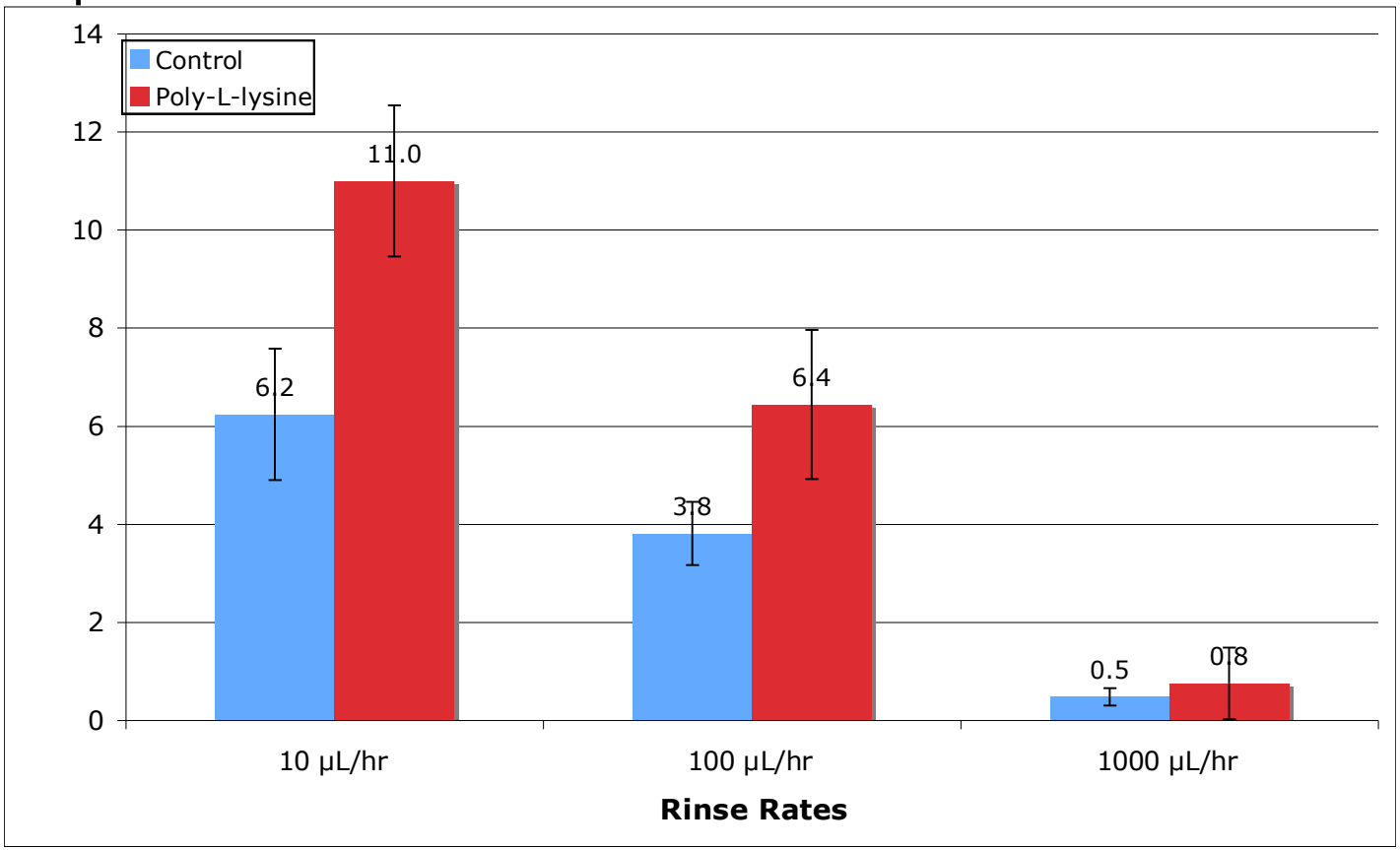
Fluorescence image



ImageJ analysis

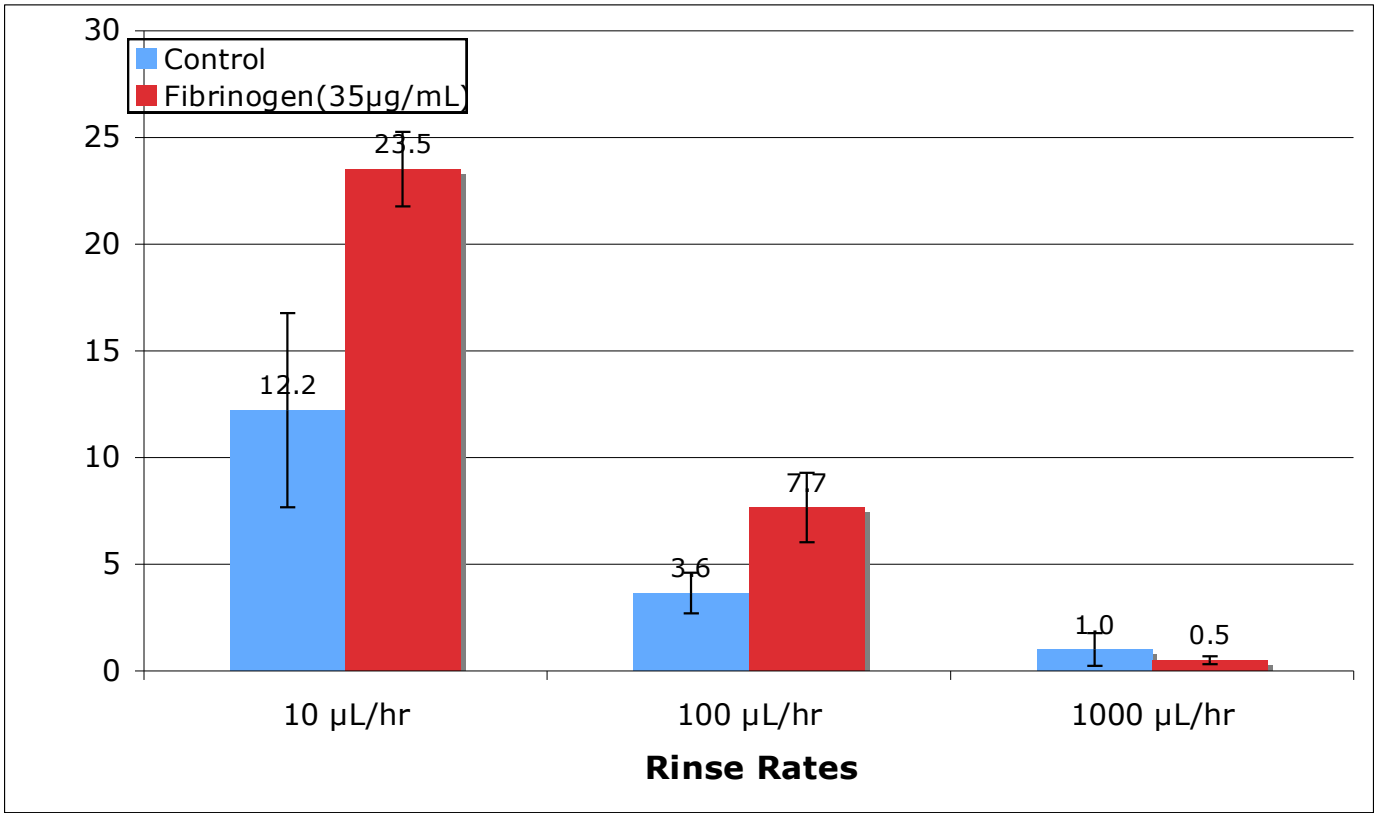
Results with poly-L-lysine (PLL)

- PLL coated channels vs. uncoated channels subjected to different rinse rates
- 0.01% PLL flow into channel, dried over night
- Bacteria of OD_{600} 1.2, incubated 2hrs at 37 °C, rinsed with LB growth media 30min at 10µL/hr



Results with Fibrinogen (FG)

- FG coated channels vs. uncoated channels subjected to different rinse rates
- 35µg/mL FG in PBS flowed into channel, incubated 1hr at 37 °C, rinsed with LB growth media 30min at 50µL/hr
- Bacteria of OD_{600} 1.2, incubated 2hrs at 37 °C, rinsed with LB growth media 30min at 10µL/hr



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Results with Varying Incubation Times

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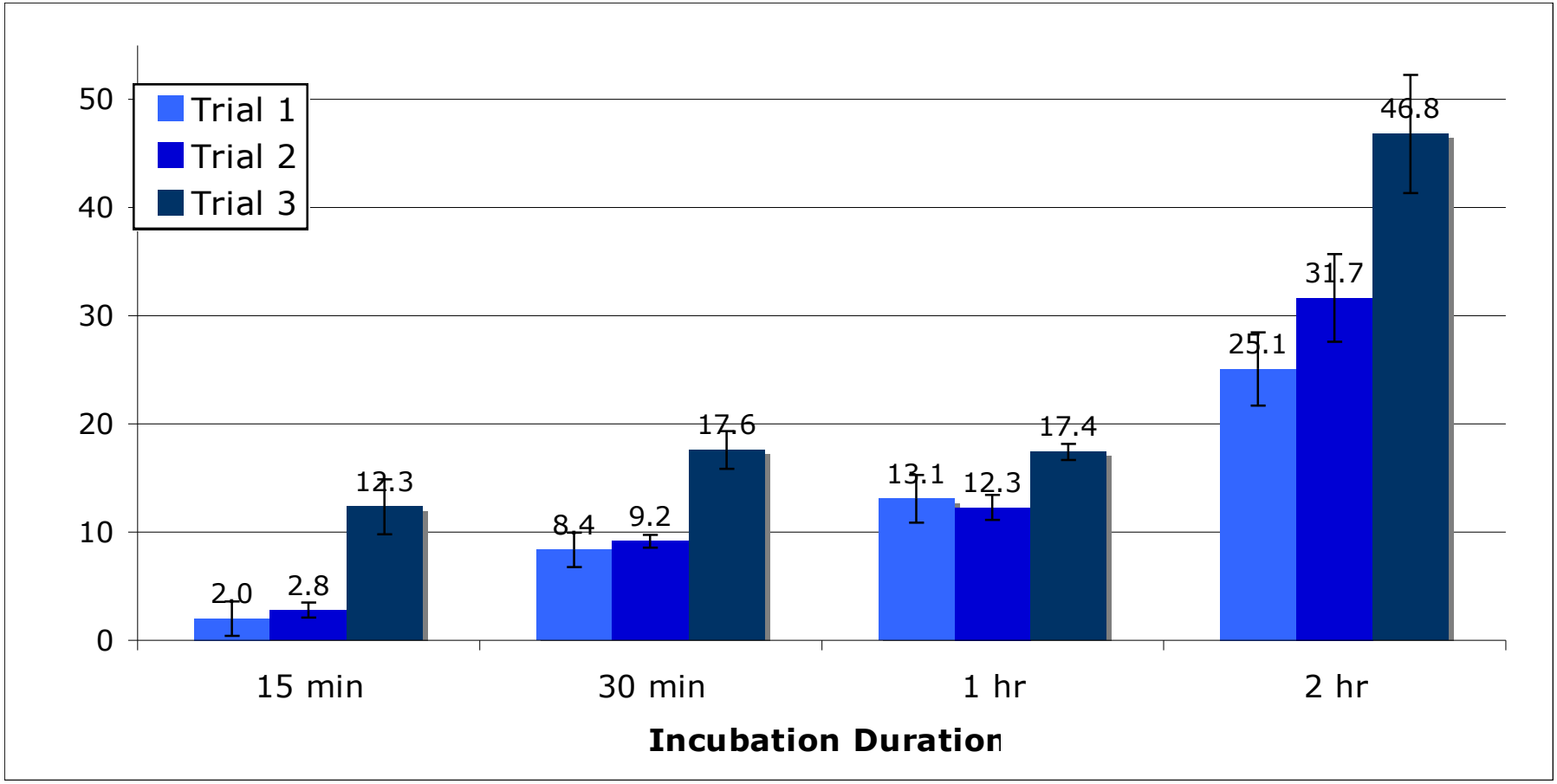
- Varying bacterial incubation times with uncoated channels
- Bacteria of OD_{600} 1.3, incubated at 37 °C, rinsed with LB growth media 30min at 10 µL/hr



Conclusion

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

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