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

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Abstract—**Formation of bacterial biofilms, composed primarily of bacterial cells and a matrix of extracellular polysaccharides that provide the microorganisms advantages such as increased antibiotic resistance, involves an initial attachment of bacteria to a surface. A device has been designed to optically monitor the growth of biofilms under microfluidic conditions. Incorporation of poly-L-lysine and fibrinogen bound to the walls of the device was utilized to increase preliminary adhesion of *Escherichia coli* BL21 to the coated surfaces by approximately 100% and 200%, respectively. Bacterial incubation duration was optimized at 2 hours. Results are expected to enhance repeatability of biofilms formed in microfluidics.**

Keywords—**microfluidics, bacterial biofilms, poly-L-lysine, fibrinogen**

I. INTRODUCTION

ONE form of cell-cell communication between single-cell organisms is a mechanism known as quorum sensing. Bacteria are known to release certain types of chemical-signaling molecules while uptaking the same molecules produced by surrounding bacteria. There are three main forms of signaling molecules used in quorum sensing; *Escherichia coli* are known to release autoinducer-2 (AI-2) which is a universal communicating molecule understood by both Gram-positive and Gram-negative bacteria.

Through quorum sensing, bacteria are able to collectively coordinate actions when a threshold population has been met. For example, bacteria exhibit a change in their phenotypes to produce compounds needed to form a bacterial biofilm, which is comprised mostly of proteins, bacteria and an extracellular polysaccharide matrix. Investigations for biofilms are highly demanded as it has been shown that they provide an increased resistance to antibiotics, in addition to the fact that 80% of all infections involve biofilms [1]. Biofilms can form on both living and non-living surfaces such as the urinary tract, respiratory tract, or foreign implants. The most important stage in the formation of a biofilm is the first stage when free-floating bacteria attach to a surface. If these bacteria do not adhere firmly to the surface, a biofilm cannot grow. Also, these first colonists on the surface can facilitate the attachment of additional bacteria.

Recently, bacterial biofilms have been studied in microfluidic conditions, providing the advantages of a highly controllable environment, amplified sensitivity, inexpensive and quick setup, and parallel operation [2]. A significant problem in the current application of microfluidics to studying biofilms is that its formation has high variability and is not consistent. In the presented work, the adhesion of *Escherichia coli* to the walls of a microfluidic channel was tested. Poly-L-lysine and fibrinogen, which have been shown to increase adhesion of other types of bacteria [3,4], is tested for increased bacterial adhesion to the microfluidic channel. The goal is to enhance the repeatability of bacterial biofilms formed in the microfluidic platform.

II. MATERIALS AND METHODS

A. Microfluidic Platform Design and Fabrication

The platform is comprised of a rectangular prism molded from polydimethylsiloxane (PDMS), with a microfluidic channel in the center with a width of 500 μm, height of 100 μm, and length of 2 cm. The PDMS is patterned in 100 μm-thick SU-8 50 (MicroChem Corp,

Manuscript received August 2, 2010. This material is based upon work supported by the National Science Foundation under Grant No. 0755224.

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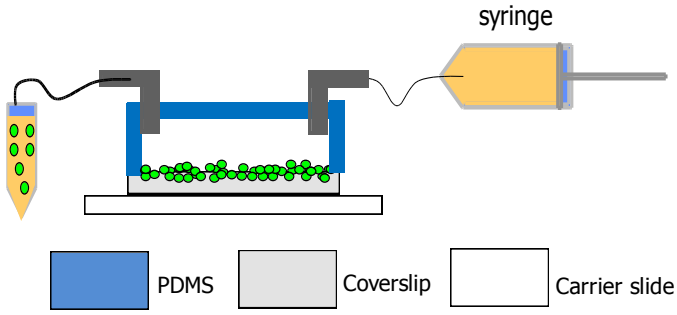


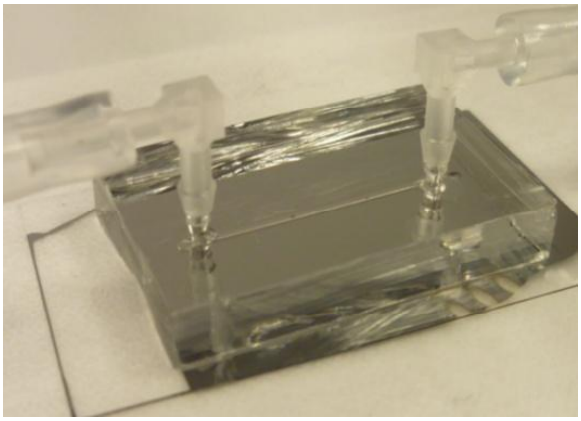


Fig. 1. a) Cross-section of microfluidic platform and fluidic interfacing
b) Microfluidic device

USA) using contact photolithography. PDMS (Sylgard 184, Dow Corning, USA), in a 10:1 ratio of resin to curing agent, with a total mass of 33g, is poured over the mold and cured at 80 °C for 20 minutes. Ports for interfacing the channel to fluidic tubing are drilled into the PDMS layer using a 2 mm dermatological punch. The molded PDMS is reversibly bonded to the chip, allowing for disassembly, cleaning, and reuse of each chip after experimentation. Methanol is applied to the PDMS layer, which is then placed onto a coverslip; evaporation of the methanol produces a reversible bond. A schematic of the microfluidic platform is given in Figure 1.

One end of Tygon tubing is connected to a PDMS port via a barbed tubing coupler (McMaster Carr 5117K41, USA), and the other is connected to a syringe pump operating in suction mode. The entire assembly is positioned in an incubator maintained at 37 °C.

B. *Strains Used*

E coli. BL21 pGFP was selected as a model organism. A plasmid encoding the green fluorescent protein (GFP) is introduced into the bacteria so that it can be marked and imaged using fluorescence microscopy. Bacteria cultures are all suspended in LB growth media.

C. *Adhesion Layer Deposition*

Devices to be coated with poly-L-lysine are first sterilized with 70% ethanol, and then 0.01% poly-L-lysine (Sigma-Aldrich, USA) is suctioned into the channel. Connectors and tubings are removed and the device is left overnight in room temperature to allow the poly-L-lysine solution to dry. On the next day of the experiment, the tubing and connectors are put back into place.

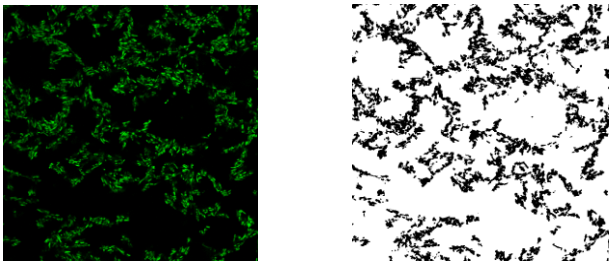
For fibrinogen-coated channels, a fibrinogen stock solution is first made by adding 25 mg of fibrinogen (Sigma-Aldrich, USA) into 10 mL of Phosphate Buffered Saline (PBS), yielding a concentration of 2.5 mg/mL. A solution of 35 µg/mL of fibrinogen made from the stock solution was used to coat the channels after sterilization. The solution is incubated in the channel for 1 hour at 37 °C, and then rinsed at 50 µL/hr.

Control channels have no coating; therefore require no additional procedures before inoculation with bacteria.

Experiments with variable incubation durations are performed on devices where the channels remain uncoated.

D. *Platform Operation and Imaging*

To inoculate the devices, bacteria grown to OD_{600} of 1.2 or 1.5 are drawn into the microfluidic channel by using a syringe connected to one end of the tubing. The bacteria are incubated at 37 °C for 2 hours, and then rinsed with LB growth media for 15 or 30 minutes at 10 µL/hr. After rinsing, connectors and tubings are disconnected from the device and a coverslip is placed on the top surface to cover the PDMS ports and prevent leakage. The device is then taken to the fluorescence microscope for imaging. The microfluidic device is flipped upside-down so that the objective lens of the microscope is closer to the bottom of the channel. Pictures are taken at 20x, ranging from three to seven images taken per channel, with an exposure time of 1 s to increase contrast in the low light intensity setting of the channel. The channels are analyzed for bacterial adhesion by calculating the amount of area covered by bacteria in each picture using ImageJ (National Institutes of Health). The images are converted into 8-bit images, inverted, and adjusted for threshold, as shown in Figure 2. Particles are measured for percent area covered and the percentages are averaged.



a) Fluorescence image b) ImageJ analysis

Fig. 2. Example of evaluating bacterial adhesion. a) Image obtained using fluorescence microscopy. b) Image produced for analysis with Image J

III. RESULTS

A. *Bacterial Adhesion Promotion Using Poly-L-lysine*

The design of the device enables multiple platforms to be run simultaneously. Figure 3 shows two trials of the same experiment operating concurrently. Devices with no adhesion promoters acted as controls for devices that were coated with poly-L-lysine. Each device was randomly sampled three times for imaging bacterial attachment to the surfaces. A bacterial OD_{600} of 1.2 was used, rinsing the bacteria for 30 minutes at 10 µL/hr after 2 hours of incubation. Error bars are the calculation of one standard deviation above and below the mean. Bacterial average percent covered in poly-L-lysine-coated channels are roughly two-fold increase from controls, as shown in Figure 3.

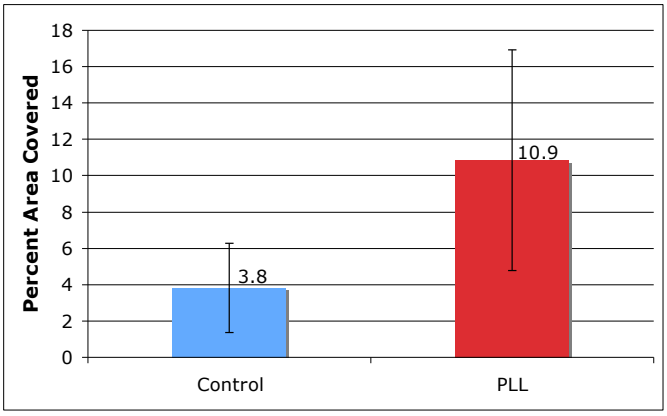


Fig. 3. 2 trials of poly-L-lysine covered channels vs. uncoated channels, 3 samples per channel

The effects of poly-L-lysine on bacterial adhesion can be further investigated by subjecting the bacteria to different flow rates during the rinsing procedure. Rinse flow rates of 10, 100 and 1000 µL/hr are used on the bacteria within control and poly-L-lysine coated channels. Referring to Figure 4, as flow rates increases from 10 µL/hr to 1000 µL/hr, bacterial coverage significantly drops in both the uncoated and the poly-L-

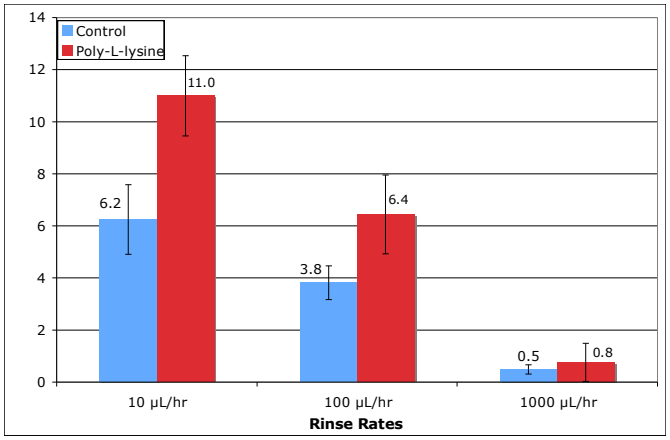


Fig. 4. Control and poly-L-lysine rinsed at different flow rates, 7 samples per channel

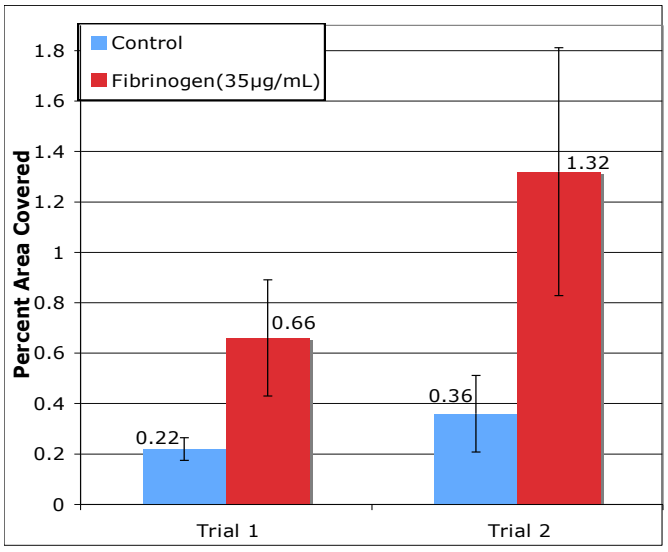


Fig. 5. 2 trials of fibrinogen covered channels vs. uncoated channels, 5 samples per channel

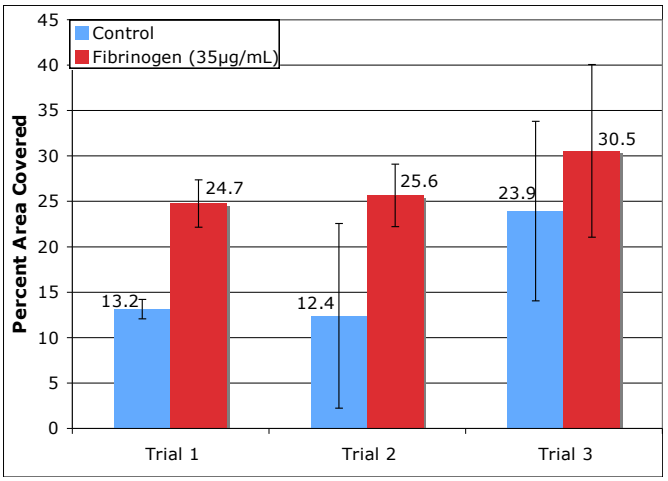


Fig. 6. 3 trials of fibrinogen covered channels vs. uncoated channels, 7 samples per channel

lysine coated channels. At 10 µL/hr and 100 µL/hr, poly-L-lysine improves bacterial adhesion by the same magnitude, whereas at 1000 µL/hr there is no difference between the two channels.

B. Bacterial Adhesion Promotion Using Fibrinogen

Another adhesion promoter that was investigated was fibrinogen at a concentration of 35 µg/mL in PBS, as suggested by concentrations found in literature [1]. Five trials of fibrinogen-coated channels compared to controls were performed on two different days with different cultures of bacteria. Figure 5 represents two trials with five samples taken per channel, with a bacterial OD_{600} of 1.5, rinsing the fibrinogen solution for 15 minutes at 50 µL/hr and rinsing the bacteria for 15 minutes at the same flow rate. Figure 6 represents three trials, completed on a separate day, with seven samples per channel, a bacterial OD_{600} of 1.2. The fibrinogen solution was rinsed for 15 minutes at 50 µL/hr while the bacteria was rinsed using the same methods as the first set of trials. Results in Figure 5 show that fibrinogen increases bacterial adhesion roughly three-fold while Figure 6 shows an increase of approximately two-fold, excluding Trial 3. In Trial 3, a smaller difference was observed, with large overlapping error bars.

It should be noted that the percentages from the results of Figure 5 are an order of magnitude smaller than usual results when compared to data collected from all other experiments, even though the OD_{600} of bacteria used in all trials were similar in magnitude.

Due to repeatedly observed abnormally high bacteria concentrations near the PDMS ports, Figure 7 represents the data from Figure 6 excluding images taken nearest to the PDMS ports. Figure 7 shows no significant changes in the values of the averages, but noticeable reduction in the error bars.

In order to allow for comparison to the experiments with poly-L-lysine, flow rates to rinse away free-floating bacterial cells from fibrinogen-coated channels were varied from 10 µL/hr up to 1000 µL/hr. Similar results to the poly-L-lysine are seen with fibrinogen as flow rates are increased. At both 10 µL/hr and 100 µL/hr fibrinogen increases adhesion rates by approximately two-fold. The error bars at 1000 µL/hr are large enough to confidently conclude that there is no difference at that flow rate.

C. Influence of Incubation Times on Adhesion

Bacterial incubation times were varied on uncoated channels to determine if incubation duration could be reduced without hindering bacterial adhesion.

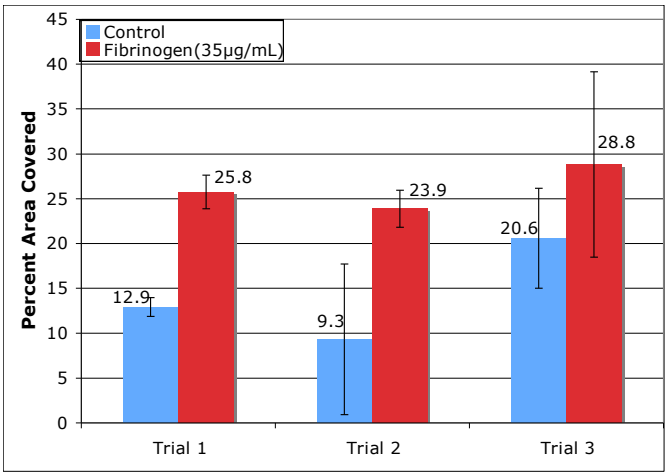


Fig. 7. 3 trials of fibrinogen covered channels vs. uncoated channels, first and last images excluded, 7 samples per channel

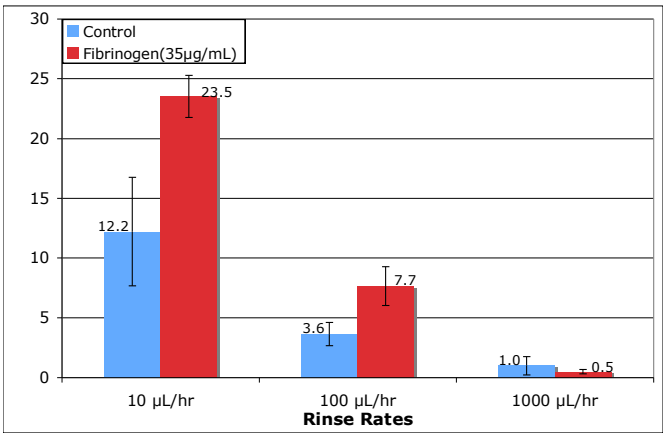


Fig. 8. Control and fibrinogen rinsed at different flow rates, 7 samples per channel

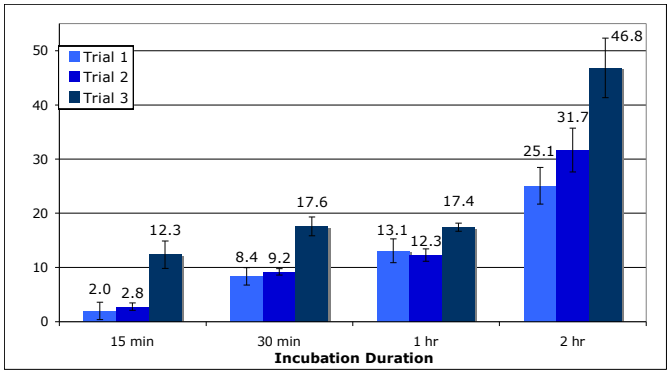


Fig. 9. 3 separate trials of uncoated channels with varying incubation durations

Procedures for preparing devices are preformed the same as preparing control devices, with only incubation times varying between 15 minutes, 30 minutes, 1 hour, or 2 hours. Three trials are completed on three separate days, each with a bacterial OD_{600} of 1.2. After inoculation, bacteria were rinsed for 30 minutes after incubation at 37°C, for 30 minutes at 10 µL/hr. Results

from the three trials are presented in Figure 9. In all trials, maximum adhesion was observed using 2 hour incubation time.

IV. DISCUSSION

The methods described present a technique to evaluate the adhesion characteristics of bacteria within microfluidic channels. When poly-L-lysine is coated along the channels, a two-fold increase in bacterial adhesion results. The large error bars in those devices suggest there is considerable variability within each channel, though it is evident that the overall average percent covered of the channel is still significantly larger than those of the controls. If more than three images per channel had been taken, it would be assumed that the error bars would decrease in size.

When different flow rates are used to rinse the bacteria after incubation, the data from both types of coated channels show that there is a significant drop in adhesion rate when flow rates are increased above 10 µL/hr. The trend is expected as higher rinse rates correspond to higher shear stresses on the bacteria, which would cause more cells to be pulled off the surfaces of the device. The results suggest that poly-L-lysine improves bacterial adhesion but only within a certain range of rising velocities; 1000 µL/hr is above the threshold by which poly-L-lysine has an effect. The experiment was performed twice (Figure 4 shows the second trial); data from first trial not shown but exhibits the same trend.

The first set of trials experimenting with fibrinogen show an increase in bacterial adhesion by three-fold while the second set of trials show an increase of around two-fold. The difference in magnitude between values in the first set and the second set can be explained by the fact that the bacteria used in the first set had been stored for one week in a refrigerator. In addition, the culture was made from bacteria that was stored in a freezer of only -15 °C, while in the second set of trials the bacteria was freshly cultured from bacteria that was kept in a different freezer set to -80 °C. It is suspected that the conditions for the first trial altered the adhesion properties of the bacteria equally in the control and fibrinogen-coated channels.

It was observed that near the PDMS ports there were abnormally high concentrations of bacteria due to increased amount of fluidic “dead space” in these regions; therefore the images taken closest to both of the ports were assumed to be not representative of the channel, and were excluded to reduce variability. The omission of the two samples does not significantly change the averages but noticeably reduces the size of the error bars.

Fibrinogen and poly-L-lysine are similar in that when exposed to higher flow rates, bacterial adhesion decreases. Fibrinogen also has a range of rinse rates where it is only effective, and as with poly-L-lysine at 1000 µL/hr improvements in bacterial adhesion are attenuated.

From the results we conclude that incubation times cannot be reduced below 2 hours. When incubation is less than 2 hours adhesion is diminished, which would not aid in increasing biofilm formation repeatability.

V. CONCLUSION AND FUTURE WORK

The effects of poly-L-lysine and fibrinogen were tested as an adhesion promoter in a unique microfluidic platform. Both molecules were shown to increase bacterial adhesion to the walls of the channels by two to three-fold when compared to uncoated channels. These results could lead to the implementation of either of these molecules in the operation of the microfluidic device to monitor bacterial biofilm growth. Through improved adhesion it is expected that biofilms formed in the microfluidic platform will be more stable and therefore more repeatable. Bacterial incubation will require at least 2 hours. Further work may include coating the channels with varying concentrations of fibrinogen and determining if other species of bacteria will give rise to the same results.

ACKNOWLEDGMENT

The authors acknowledge financial support from the R. W. Deutsch Foundation and the National Science Foundation Emerging Frontiers in Research and Innovation (NSF-EFRI).

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