Bacterial Mechanics on a Chip

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Abstract:

The ability of bacteria to squeeze into constrictions a fraction of their size can lead to bacterial infections such as osteomyelitis. In osteomyelitis, bacteria cells squeeze into small channels in the bone where they are protected from immune cells and become much more difficult to treat with antibiotics. To simulate the mechanical loading conditions bacteria experience as they move through sub-micron channels, bacteria were analyzed in similarly sized tapered channels through the fabrication of a microfluidic device. The sub-micron channels were created using deep ultraviolet (DUV) photolithography in the CNF, and then etched to transfer the design to a fused silica device. The design includes an inlet of approximately 1.2 µm, an outlet of 250 nm, and is 75 µm long. The bacteria trapped in the channels are imaged with both traditional and super-resolution microscopy. The distance travelled is then measured and compared to the pressure drop experienced by the bacteria in the channels. This pressure drop is defined as the difference in pressure across the bacteria trapped in the channel. Data collected this summer revealed that under a high inlet pressure, the bacteria can go into a channel width a fraction of their size. At a low inlet pressure, the bacteria will not squeeze into a constriction less than half their diameter. However, with the introduction of a large pressure drop, the bacteria can squeeze into a constriction up to a quarter of their diameter (Figure 1).

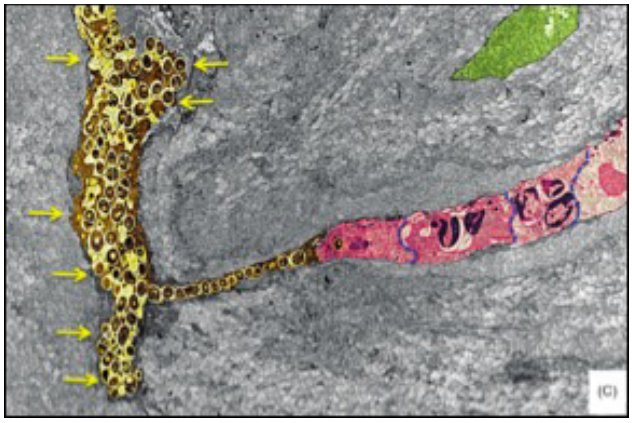

Figure 1: MRSA (yellow) penetrating bone (gray) to point where immune cells (pink) can't reach the MRSA (Nishitani, et al. 2016).

Summary of Research:

Extrusion loading is the loading mechanism the lab refers to as the mechanical stimuli that *E. coli* undergo when in the designed tapered channels. Extrusion loading is unique among methods of mechanically stimulating bacteria in that it achieves a non-uniform stress and strain experienced by the bacteria.

Manufacturing the microfluidic device begins with a CAD design (Figure 2). The channels in the design are tapered, ranging from an inlet width of 1.2 µm and an outlet width of 250 nm. The design is created so there would be varying pressure drops across each channel. This varying pressure drop is because of a resistance to fluid flow in the bypass (Figure 3). There is a decline in

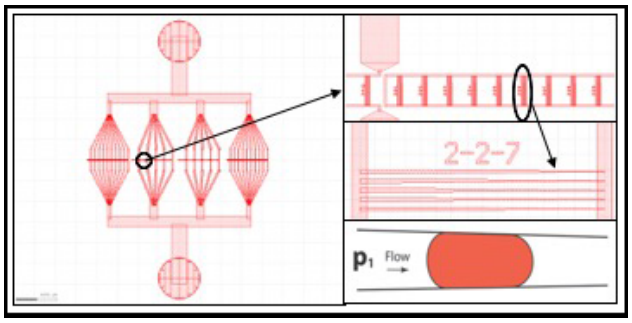

Figure 2: The tapered channels used to trap bacteria have a 1.2 µm inlet, a 250 nm outlet, and are 75 µm long. Multiple tapered channels are on each device. Six devices are present on each wafer.

pressure as the fluid moves through the channels, giving every channel its own distinct upstream pressure. The device uses 600 different channels so that several different pressure drops can be examined at once.

The silica glass device is created with DUV lithography. Fused silica was chosen because it is much stiffer than the bacteria and its transparency allows for microscopy. SEM, AFM, and the profilometer are used to check for proper taper geometry, outlet width, channel depth and to allow optimization of the process in subsequent application. After characterization, the device is bonded to a thin (170 nm) silica wafer and ready for experimentation. The device is placed on the microscope stage and bacteria in liquid suspension are flowed into the inlet using a syringe pump with a pressure gage reporting pressure at the inlet.

Experiments are performed at inlet pressures of 25 kPa and 60 kPa. Using a syringe pump that controls flow rate, the bacteria in M9 media are transported into the device. A pressure sensor is connected to the tube and a computer so that the pressure can be monitored and controlled based on the flow rate of the syringe pump.

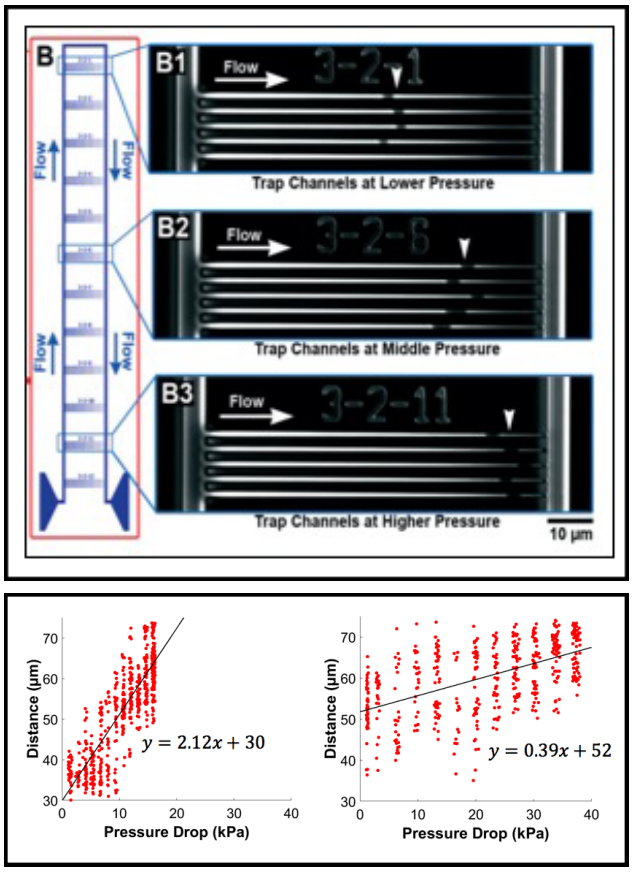

Figure 3, top: Tapers are placed in sets of five separated by a distance. Each set of tapers experiences a different drop in pressure across its length. This pressure drop is determined by the difference between upstream and downstream pressure (Sun et al Lab Chip 2014). Figure 4, below: Shown here are over 1000 analyzed cells. The relationship between pressure drop across the channel and penetration of the bacteria differs between an external pressure of 25kPa (left) and an external pressure of 60 kPa (right).

Images of the bacteria in the tapered channels are collected using transmission microscopy and the distance traveled by the bacteria was determined. The pressure drop across each bacterium in a tapered channel is determined using hydraulic circuit calculations. The distance traveled by bacteria is determined using the channel's number labels as a positional marker in the experimental images. The results indicate the relationship between loading conditions and how far the bacteria travel.

Results and Conclusions:

Over 1000 cells from over 20 different experiments were analyzed. Experiments were performed at both 60 kPa and 25 kPa inlet pressure. At a 60 kPa inlet pressure, the regression line shows an intercept of 52 µm traveled (75 µm channel length), and an average width of 500 µm under a 1 kPa pressure drop; R^2 = 0.39. At a 25 kPa inlet pressure, the regression line shows an intercept of an average of 30 µm traveled; R^2 = 0.71. In regards to reaching max constriction (250-300 nm) in the channels, this can be done in the 60 kPa experiments at approximately 6 kPa pressure drop, while it takes at least double that in the 25 kPa conditions.

It was previously undetermined whether in the 25 kPa experiments the bacteria would be able to reach the end of the channels where it is under the max constriction. Therefore, these findings present the lab with new information regarding loading conditions that enable bacteria to squeeze deep into these channels (Figure 4).

Future Work:

Future work would be to conduct a similar experiment, but with bacteria known to cause infections, such as *Staphylococcus aureus*. Since this strain of bacteria has a higher safety level than the *E. coli* used, the study would have to be moved to a different lab facility. As more of a long-term goal, we want to find out what component or components of bacteria play the biggest role in how they are able to squeeze into these sub-micron channels. Finding the biological pathways in which bacteria squeeze into these sub-micron channels could eventually lead to better antibiotics for treating bacterial infections.

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