Investigating the Effect of Environment Shape on Bacteria Growth at the Microscale

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Primary CNF Tools Used: ABM contact aligner, Hamatech wafer processor develop 1, P10 profilometer

Abstract:

***Escherichia coli* (*E. coli*) bacteria are sensitive to pressures exerted by their physical environment. The constraints that differently shaped growth chambers have on cell populations over time are a significant extension of this fact and play a part in characterizing bacteria growth. By utilizing the resources of the Cornell NanoScale Facility, we created a microfluidic device that features a large central fluid distribution chamber and hundreds of tiny growth chambers designed to grow bacteria at a 1 µm height display to view different test geometries and analyze their growth patterns. The device was produced by spinning micrometer thick layers of negative photoresist onto a silicon wafer and exposing to create a pattern designed in L-Edit CAD software. The wafer served as the mold for the actual device, which we then cast in PDMS. This created reproducible devices with channels for bacteria and nutrients to flow through and grow. A type of *E. coli* was genetically engineered to produce fluorescent bacteria that don't produce biofilms and were grown separately before being injected into the device. The results of this experiment play a part in widening the pool of knowledge for under what conditions bacteria thrive or stagnate, crucial data towards solving World Health Organization global health challenges such as antimicrobial resistance.**

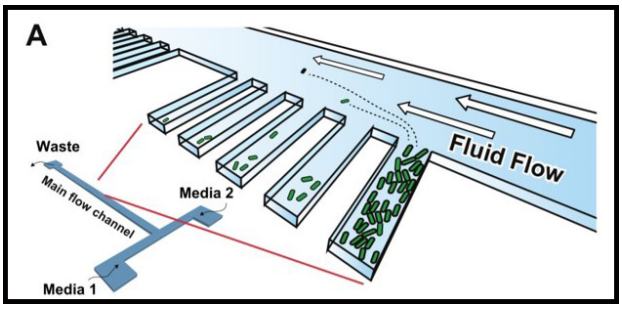

Figure 1: Microfluidic device to culture E.Coli.

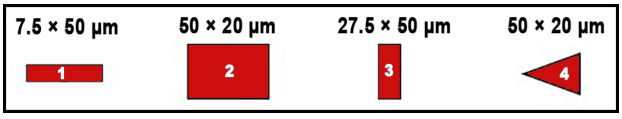

Figure 2: Geometries of four devices on the fluidic chip.

Characterizing bacteria growth is one of the primary ongoing objectives of biological research. By expanding the pool of information available about how bacteria grow in different environments, application-based research on bacterial diseases, biofuels as an alternative to fossil fuels, and plasmid genetic engineering is optimized.

Summary of Research:

We chose to focus on characterizing how *E. coli* bacteria responds to environmental conditions, specifically how the geometry containing the starting sample of bacteria limits or bolsters the growth of bacteria over time. In this preliminary qualitative research, we utilized the resources of the Cornell NanoScale Facility to produce a microfluidic device, a device that would allow us to monitor bacteria growth in growth chambers one micrometer thick, while being pumped nutrients through a pneumatic system.

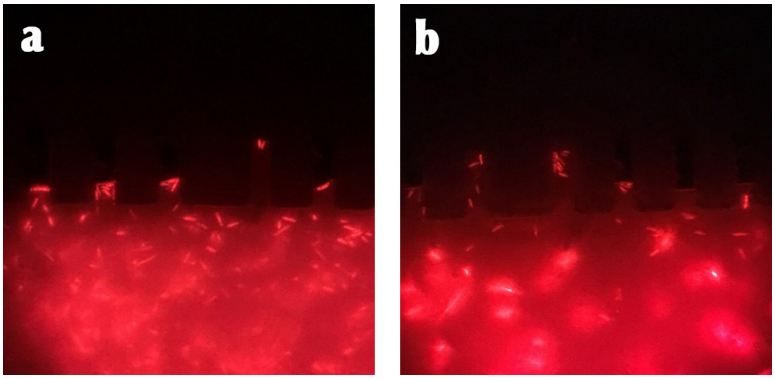

Figure 3, a and b: Fluorescent bacteria grow in Device 1 after two hours.

This microfluidic device provides a unique environment in which the bacteria and nutrients exhibit non-laminar flow, meaning that these structures can be assessed on their contribution to osmosis-based interaction with nutrients and bacteria.

The microfluidic device was fabricated by developing two layers on a silicon wafer. The first layer contained a one micrometer thick etching of the entire design, notably including four growth chambers with different geometries. The first layer was purposely thin, so that the bacteria growth could be viewed efficiently, and the only effect on bacteria diffusion was the basic two-dimensional geometry.

To produce this first layer, negative AZ2020 photoresist was spun onto the four-inch silicon wafer for an even coating of resist, and then the wafer was baked at 110°C, cooled, and then exposed to a contact aligner for 3.5 seconds for a total energy exposure of 41 mJ of energy. The wafer was then baked once more and run through the Unaxis 770 deep etch and Aura 1000 resist strip to complete the etching.

The second layer was 25 μm thick, and alternatively, we spun 2020 SU-8 photoresist on top of the wafer, which was baked at 95°C, exposed for 12 seconds for 140 mJ of energy, and then developed with SU-8 developer, completing the device. The second layer extended the height of the main flow channel.

The device itself was a long T-shape in which bacteria and nutrients could flow starting at the top of the T and exited through the waste at the end of the channel. Along the main channel were a couple hundred growth chambers for each of the four geometries: a long skinny rectangle with dimensions 7.5 × 50 μm (device 1), a rectangle with dimensions 50 × 20 μm (device 2), a thick but shallow rectangle with dimensions 27.5 × 50 μm (device 3), and an isosceles triangle with dimensions 50 × 20 μm (device 4). Device 4 was specifically designed to study the effects of a growth chamber with smaller surface area for starter bacteria to grow than the surface area exposed to the main channel.

When hypothesizing the growth chamber geometries that would result in the highest change in cell count over time, we decided that the two largest contributing factors to the success of the designs would be the amount of surface area at the back of the device and the amount of surface area exposed to the main channel. This idea was grounded in the observation of similar microfluidic device bacteria growth experiments, where a few starter bacteria stay at the back of the device and serve as the main progenitors of new bacteria over the course of the trial period.

From the qualitative results we received in the form of pictures before the experiment began and two hours after, Device 1 was the only one to experience major growth, while Devices 2 and 4 had a net loss in bacteria, and Device 3 was too malformed to draw conclusions. We expected Device 4 to have poor growth due to its combination of low surface area at the back of the device and high surface area facing the main channel, but we were surprised at the lack of growth from Device 2 compared to the high growth in Device 1. We had overestimated the importance of surface area for growth at the back of the device versus the adverse effects of diffusion.

Acknowledgements:

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