Microfluidics Channels for Zinc Metal Homeostasis

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Principal Investigator(s): Peng Chen

User(s): Felix Alfonso

Affiliation(s): Department of Chemistry and Chemical Biology, Cornell University

Primary Source(s) of Research Funding: National Institute of Health, National Institute of General Medical Sciences

Contact(s): pc252@cornell.edu, fsa33@cornell.edu

Website: http://chen.chem.cornell.edu/

Primary CNF Tools Used: Heidelberg mask writer - DWL2000, SÜSS MA6-BA6 contact aligner, Oxford Cobra ICP etcher, Plasma-Therm deep silicon etcher, P7 profilometer

Abstract:

We constructed a custom-made microfluidic device for the control growth of *Escherichia coli* (*E. coli*) colonies in microchambers. The confinement of the cells is achieved by matching the height of the microchambers with the diameter of the *E. coli* cells. The objective of our study is to image *E. coli* strain with fluorescent protein reporters to elucidate the role individual cells play in colonies achieving metal homeostasis.

Summary of Research:

Biological processes in the gut microbiome heavily depend on the harmonious balance between microbial communities and a host. This balance is maintained by chemical and biophysical cues that are exchanged between organisms to coordinate behavior. Zinc is an essential micronutrient for all living organisms [1]. It plays a vital role in protein folding, catalysis, and gene regulation [2]. To regulate the uptake/efflux of metal ions, bacterial cells control the transcription of the protein pumps with metal-responsive transcription regulators that sense the cellular concentration of metal ions.

The purpose of this project is to quantify the management of Zn^{2+} in a microbiome and determine the role the individual cells have in the colony achieving metal homeostasis. As a model system, *Escherichia coli* (*E. coli*) will be used to study community-derived zinc metal regulation. *E. coli* cell's motility and poor adherence to a substrate make it difficult to conduct imaging studies with long time scales. Microfluidics technology is a widely accepted method to study bacterial communities in a controlled environment [3]. A microfluidic platform permits tight control of the nutrients influx and has been successfully used for long-timescale imaging studies [4].

The design of the microfluidic device is shown in Figure 1. This study focuses on community behavior; thus, we chose the width and length of the chamber to be about ~ 200 μm. The depth of the microchamber has been chosen to match the diameter of an *E. coli* cell (~ 1 μm) [5]. The high aspect ratio (200:1) is problematic due to the possibility of ceiling collapse [6] caused by the attractive forces between PDMS and the glass coverslip. Therefore, the roof is supported by regularly spaced pillars. The regularly spaced pillars were designed to have the shape of circles or squares with a diameter or length of 4 or 8 μm.

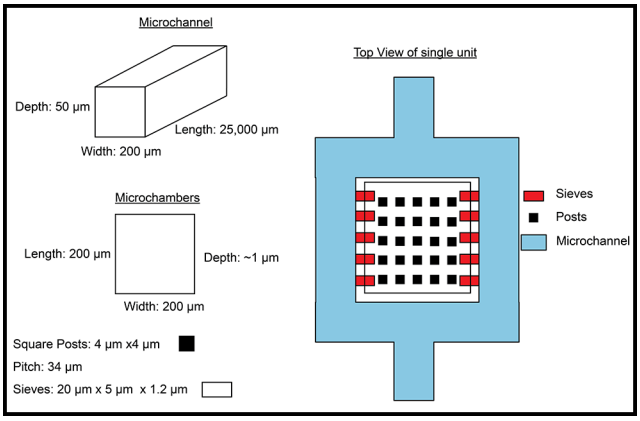


Figure 1: Schematic of the microfluidic device design of the microchannel and microchambers with the desired dimensions.

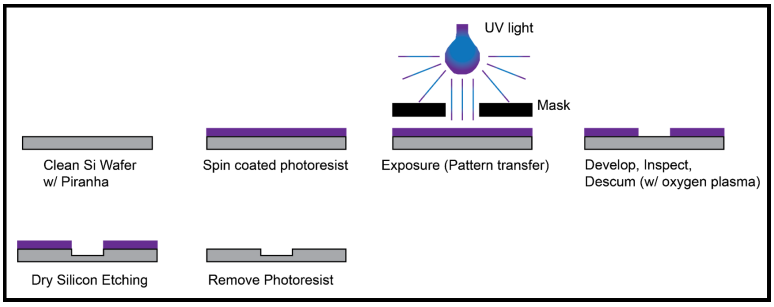
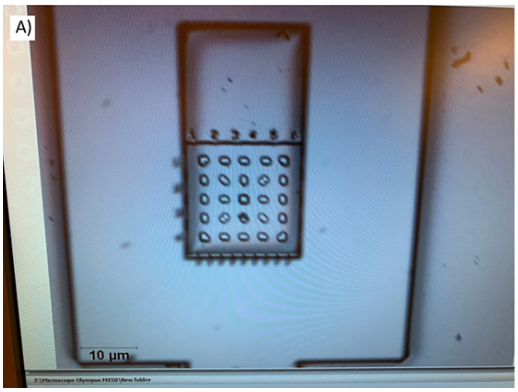


Figure 2: Fabrication scheme of the silicon mold for the microfluidic device.



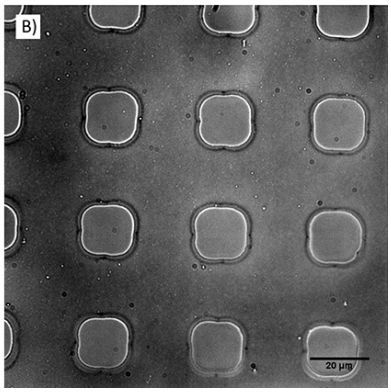


Figure 3: A) A photographed image of a prototype of the microfluidic device. B) Bright-field image of the PDMS posts used to prevent ceiling collapse.

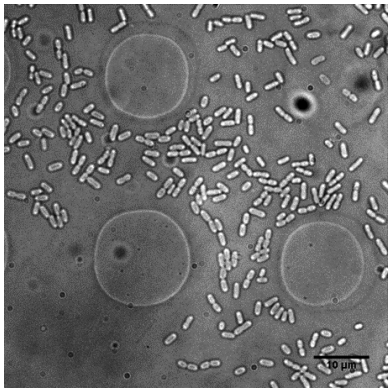


Figure 4: A bright-field image of one growth chamber filled with E. coli *cells.*

The microfluidics devices are constructed using well-established silicon nanofabrication technology. The fabrication scheme is summarized in Figure 2. Briefly, silicon wafers were cleaned with piranha solution from the Hamatech wafer processor. Afterwards, they were spin coated with photoresists. Photoresist was removed 2 mm from the edge of the wafer using the edge bead removal system. The substrate was patterned using a pre-patterned photomask made using the Heidelberg mask writer - DWL2000. The SÜSS MA6-BA6 contact aligner was used for the UV light exposure of the wafer. After developing the wafer and cleaning it with a brief oxygen plasma. The chamber was created by etching about ~ 1 μm of silicon using the Oxford Cobra ICP etcher. The photoresist was removed using the photoresist stripper bath. The height of the chamber was measured using a profilometer. The same process was repeated to create the flow channels; however, the Plasma-Therm deep silicon etcher was used for the etching step to create channels with depths of about 50 μm.

The final step is coating the silicon mold with a hydrophobic molecular monolayer such as tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (FOTS). An image of the prototype of the microfluidic device is shown in Figure 3A. After casting PDMS on the silicon mold, the microfluidic devices were bonded to coverslips and inspected using a microscope. The brightfield image of the chamber shows a regularly spaced PDMS post preventing the collapse of the ceiling (Figure 3B).

The loading of the cells into the chambers is a challenge. One approach is to increase the gauge pressure inside the devices to inflate the microchannels, and cause the ceiling to bulge up, increasing the height of the channels and allowing the passage of the cells. Reducing the pressure causes the channels to deflate, and the ceilings return to their original height. The brightfield image shown in Figure 4 shows an example of a chamber loaded with *E. coli* using the procedure described previously. The future step is to test this prototype and optimize the conditions for the successful completion of the project.

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