Building Microfluidics Devices to Study Zinc Metal Homeostasis in *E. Coli* Communities

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Primary CNF Tools Used: Heidelberg Mask Writer DWL2000, SUSS MA6-BA6 Contact Aligner, Oxford Cobra ICP Etcher, Plasma-Therm Deep Silicon Etcher, P7 Profilometer

Abstract:

Microbial life has a set of molecular tools to import essential nutrients from the surrounding environment and when necessary, efflux the excess to prevent harmful toxicity. The aim of this study is to elucidate the role individual bacterial cells play in achieving metal homeostasis at the community level. With this in mind, we engineered a custom microfluidic device that facilitates the controlled growth of *Escherichia coli* (*E. coli*) colonies within well-defined microchambers. These chambers' dimensions are carefully matched to the diameter of *E. coli* cells, thereby allowing for strategic cellular confinement — a critical aspect of our investigative method. The microfluidic devices we employed exhibit dynamic environmental control features, enabling us to switch solutions rapidly for stable, pulse, or step-function nutrient exposure as well as the creation of nutrient gradients. We employed advanced molecular biology techniques to engineer *E. coli* strains equipped with fluorescent protein reporters. This unique genetic modification allowed us to visualize and quantify gene expressions linked to the intricate influx and efflux ion channels, with particular emphasis on those specific to zinc. The insights derived from this study could have profound implications for our understanding of microbial ecosystems and their interactions with the environment.

Summary of Research:

Zinc, a fundamental micronutrient, is indispensable for all living organisms [1]. It serves vital functions in protein folding, catalysis, and gene regulation [2,3]. An imbalance in zinc levels, either a deficiency or an excess, can trigger substantial alterations in the gut microbiome, thereby resulting in adverse health conditions [4,5]. Microbes have developed molecular mechanisms to efficiently import nutrients from the environment and efflux under excess. To regulate these efflux pumps, bacteria modulate the transcription of protein pumps using metal-responsive transcription regulators. These regulators monitor the cellular concentration of metal ions, guiding cells towards achieving an optimal state of metal homeostasis.

Our project is designed to explore and quantify the management of Zn2+ within a microbiome, shedding light on the role individual cells assume in establishing community-wide metal homeostasis. We have chosen *Escherichia coli* (*E. coli*) as our model organism to investigate the complexities of community-derived zinc metal regulation. E. coli's natural motility and poor adherence to substrates present challenges for long-duration imaging studies. However, microfluidics technology offers an effective solution, providing a controlled environment conducive to studying bacterial communities [6]. A microfluidic platform permits tight control of the nutrients influx and has been successfully used for long-timescale imaging studies [7]. The microfluidic device employed in our study is engineered

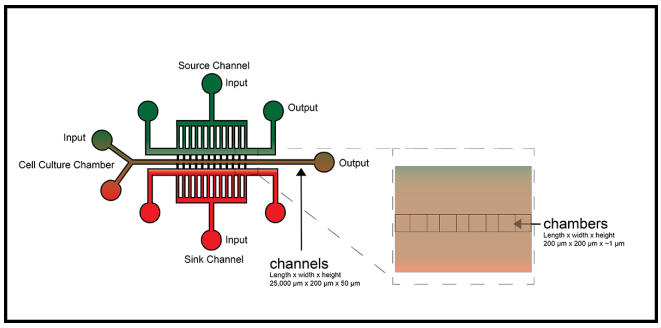

Figure 1: Schematic of gradient generating microfluidic device showing the source and sink channels and the chamber.

with three key components: a gradient generator, channels, and microchambers. The microchambers' depth is tailored to match the diameter of an E. coli cell (~ 1 μm), thereby facilitating the efficient confinement of colonies. The gradient generator was designed with the goals of creating a stable concentration gradient and minimization of convection inside the chambers. The design presented by E. Bernson, and A. Shamloo provided an optimal method to create a stable gradient inside the chamber (Figure 1, 8-9). This is accomplished by having a source chamber and a sink chamber. The molecule of interest diffuses through the micro capillaries that connect the source and sink to the main culture chamber.

The construction of these microfluidic devices relies on established silicon nanofabrication technology. The fabrication scheme is summarized in Figure 2. The fabrication process begins with silicon wafers being cleaned with piranha solution. They are then coated with photoresists, which are removed 2 mm from the wafer's edge using an edge bead removal system. The substrate is patterned using a pre-designed photomask created with the Heidelberg Mask Writer DWL2000. The Karl SUSS MA6-BA6 contact aligner provides UV light exposure for the wafer, which is developed and cleaned with a brief oxygen plasma treatment. Chambers are created by etching approximately ~ 1 μm of silicon using the Oxford Cobra ICP Etcher, and then the photoresist is removed with a stripper bath. The chamber's height is measured using a profilometer. Channel construction involves the use of SU-8 lithography, where SU-8 is spin-coated onto the substrate and patterned with the Karl SUSS MA6-BA6 contact aligner. After a curing

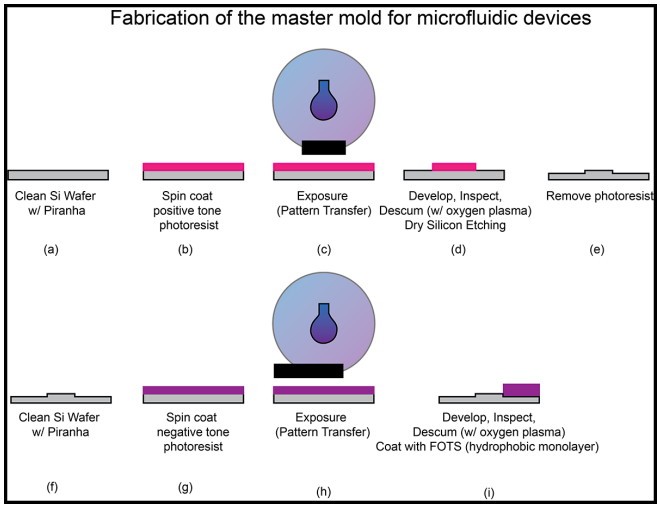

Figure 2: Fabrication of microfluidic devices combining dry etching to construct the chambers and SU-8 lithography to construct the channels.

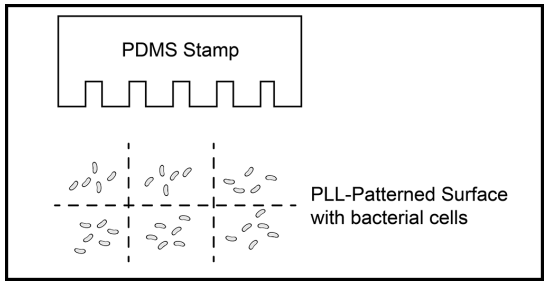

Figure 3: Patterning of the coverslip surface using PDMS stamps coated with PLL.

phase on a hot plate at 95°C, the unpolymerized SU-8 is removed with the developer. The resulting structure is then hard baked for 10 minutes at 200°C. The final step entails coating the silicon mold with a hydrophobic molecular monolayer such as tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (FOTS) to facilitate PDMS removal. After casting PDMS on the silicon mold, the microfluidic devices are bonded to coverslips and inspected under a microscope. Cells are loaded into the chambers and imaged with a microscope equipped with the appropriate laser line and filters. Currently, we are exploring new methods to load the bacterial cells into the microchambers such as patterning the surface of the coverslip using PDMS stamps coated with PLL (Figure 3). The overall goal is to pattern the surface with cells to match the geometry of microfluidic devices to achieve better spatially distributed cells.

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