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

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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:

Microbial life has evolved a set of molecular tools to import the necessary nutrients for survival from their environment and efflux the excess to avoid toxicity. The aim of this study is to elucidate the role individual bacterial cells play in achieving metal homeostasis at the community level. Hence, we constructed a custom-made microfluidic device for the controlled 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. Using molecular biology, *E. coli* strains were constructed with fluorescent protein reporters to quantify the gene expression of the influx and efflux ion channels specific to zinc. The dynamic environmental control in microfluidic devices allows us to probe how the community of bacteria achieves zinc metal homeostasis.

Summary of Research:

Zinc is an essential micronutrient for all living organisms [1]. It plays a vital role in protein folding, catalysis, and gene regulation [2,3]. Zinc deficiency or excess is associated with drastic changes in the gut microbiome, which results in poor health [4,5]. Microbial life has evolved a set of molecular tools to import nutrients from their environment and efflux excess nutrients to avoid toxicity. To regulate the efflux pumps, bacterial cells control the transcription of the protein pumps by using metal-responsive transcription regulators that sense the cellular concentration of metal ions to achieve a state of metal homeostasis.

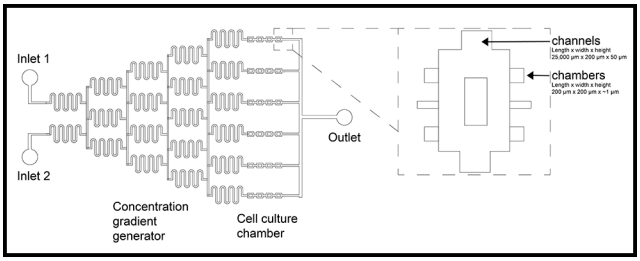

Figure 1: Schematic of the microfluidic device design of the gradient generator, microchannels, and microchambers with the desired dimensions.

The aim of this project is to quantify the management of Zn^{2+} in a microbiome and determine the role individual cells play in achieving metal homeostasis as a community. As a model system, *Escherichia coli* (*E. coli*) will be used to study the 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 [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 used in this study consist of three necessary components: a gradient generator, channels, and microchambers (Figure 1). The depth of the microchamber has been chosen to match the diameter of an *E. coli* cell (~1μm) thus facilitating the confinement of the colonies [8]. 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. Afterward, they

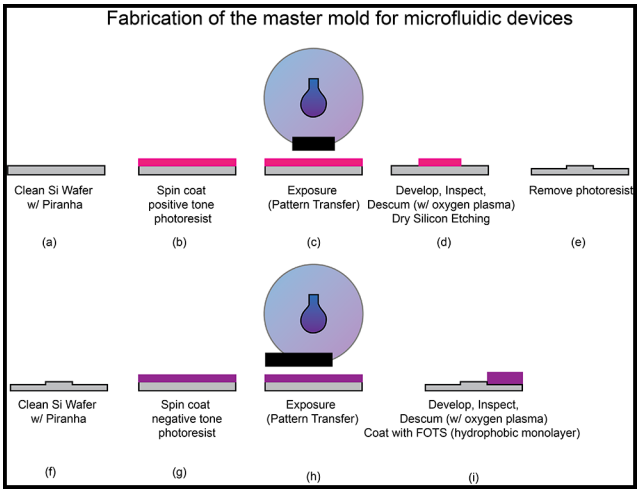

Figure 2: Fabrication of microfluidic devices combining dry etching to construct the chambers and SU-8 lithography to construct the channels.

were spin-coated with photoresists. The 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 channels were constructed using SU-8 lithography.

Briefly, SU-8 was spin-coated onto the substrate and pattern using the SÜSS MA6-BA6 contact aligner. The SU-8 was cured on a hot plate at 95°C. The unpolymerized SU-8 was removed with the developer and the resulting structure was hard baked for 10 minutes at 200°C.

The final step was 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 were bonded to coverslips and inspected using a microscope.

The cells were loaded into the chambers and imaged using a microscope equipped with the appropriate laser line and filters. The composite image shown in Figure 3 shows an example of a chamber loaded with *E. coli*. The cells colored in green are bacterial cells expressing the zinc efflux channels while the cell colored in red indicates the expression of the zinc influx channels.

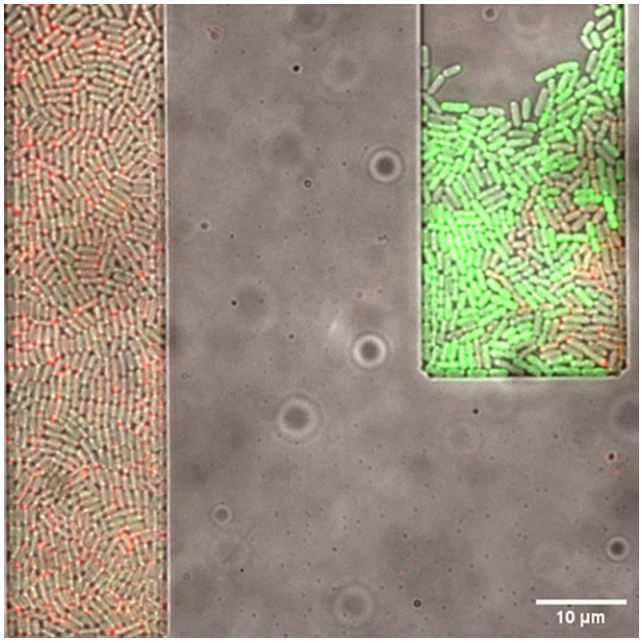

Figure 3: A composite image of E. coli *cells residing in the microchambers expressing influx ion channels (red) and efflux ion channels (green).*

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