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

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Abstract:

Bacterial organisms have developed sophisticated biochemical mechanisms to absorb vital nutrients from their surroundings while expelling surplus materials to avoid toxic accumulation. This research seeks to understand how single microbial units contribute to maintaining metal equilibrium within larger bacterial populations. To accomplish this objective, we created a specialized microfluidic system that enables precise cultivation of Escherichia coli colonies in carefully designed microscopic compartments. The size of these compartments was deliberately calibrated to match E. coli cell dimensions, creating controlled spatial restrictions that serve as a fundamental component of the experimental approach. The microfluidic platforms utilized in this work provide sophisticated environmental regulation capabilities, offering exceptional opportunities to examine and comprehend microbial behavior. Through this technology, we could examine in detail how these microscopic bacterial communities maintain equilibrium in their zinc metabolism processes. We utilized cutting-edge genetic engineering methods to develop E. coli variants containing luminescent protein indicators. This specialized genetic alteration enabled the observation and measurement of the activity of genes associated with complex ion transport systems, particularly focusing on zinc-specific pathways. The findings from this investigation may significantly advance our comprehension of microbial communities and their environmental relationships.

Summary of Research:

As an essential trace element, zinc plays a critical role in the survival of all life forms (1). This micronutrient performs crucial tasks in enzymatic processes, protein structure formation, and transcriptional control (2,3). When zinc concentrations become unbalanced significant disruptions occur in intestinal microbial communities, leading to detrimental health outcomes (4, 5). Throughout evolutionary history, microorganisms have evolved sophisticated molecular systems that enable efficient nutrient uptake from their surroundings while simultaneously expelling surplus amounts to avoid cellular damage. Bacterial cells control these export mechanisms by regulating the production of transport proteins through metal-sensitive transcriptional controllers. These regulatory elements monitor intracellular metal ion concentrations, directing cellular processes toward optimal metal balance. This research aims to investigate and measure zinc ion (Zn^{2+}) management within microbial communities, illuminating how single bacterial cells contribute to maintaining metal equilibrium across entire populations. We selected Escherichia coli as our experimental model to examine the intricate mechanisms of community-based zinc regulation. The inherent mobility of E. coli and its weak surface adhesion properties create obstacles for extended microscopic observation studies. Nevertheless, microfluidic technology provides an elegant solution by creating controlled experimental conditions suitable for bacterial community research (6). These microfluidic systems enable precise regulation of nutrient delivery and have proven successful in long-term imaging investigations (7).

Our experimental microfluidic apparatus incorporates two essential elements: flow channels, and microscopic cultivation chambers. The depth of these cultivation chambers is specifically designed to correspond with E. coli cell diameter (approximately 1 micrometer), enabling effective bacterial colony containment.

In Figure 1, we can observe a single layer of E. coli cultivated under 10 micromolar zinc conditions. The red marking indicates the chamber boundary, within which support pillars are positioned to prevent structural collapse while serving as reference points for distance

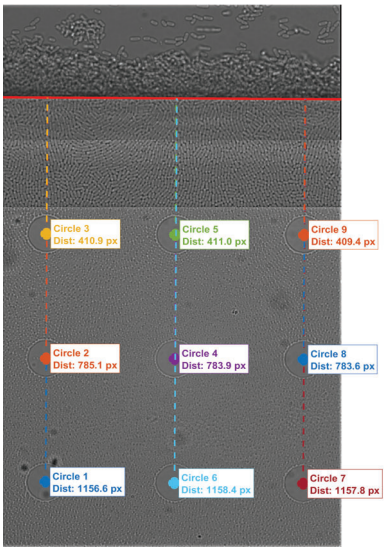

Figure 1: Monolayer of E. coli cells grown in microfluidic microchamber under 10 micromolar zinc exposure. The red line indicates the beginning of the chamber. Support posts within the chamber prevent ceiling collapse and serve as fiducial markers for distance calibration.

measurements from the pillar to the chamber opening. Through time-lapse fluorescence microscopy, we can monitor the activation of the ZntA efflux protein during continuous exposure to 10 micromolar zinc concentration. The resulting curve displays initial S-shaped kinetics followed by sharp increases in later time periods (Figure 2). By utilizing spatial coordinates from the reference markers, we can organize our data according to distance from the channel entrance, providing insights into how gene expression varies with spatial position. This visualization is achieved through a two-dimensional histogram plotting distance (y-axis) versus time (x-axis) with concentration values as binned data (Figure 3). This microfluidic platform enables comprehensive spatial-temporal analysis of efflux protein and channel gene expression, potentially establishing a foundation for understanding metal homeostasis mechanisms and developing therapeutic strategies that target bacterial metal regulation systems.

Microfluidic device fabrication uses standard silicon nanofabrication. Silicon wafers are cleaned with piranha solution, coated with photoresist, and patterned using a custom photomask and Karl SUSS MA6-BA6 Contact aligner. Chambers are etched ~1µm deep using Oxford Cobra ICP Etcher. Channels are formed via SU-8 photolithography, cured at 95°C, then hard baked

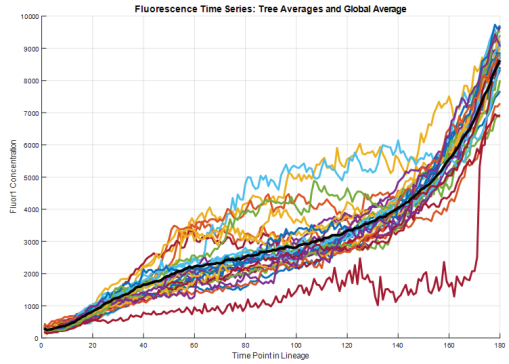

Figure 2: Time-course induction of ZntA efflux protein (nanomolar) expression under constant 10 micromolar zinc exposure measured by time-lapse fluorescent microscopy.

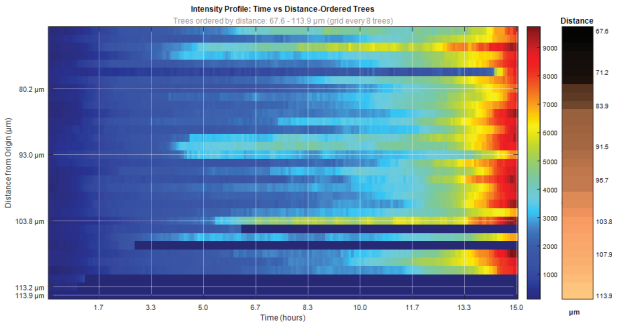

Figure 3: Two-dimensional histogram showing ZntA protein concentration (nanomolar) as a function of distance from channel entrance (y-axis) and time (x-axis).

at 200°C for 10 minutes. The silicon template is coated with FOTS for easy PDMS removal. Completed devices are bonded to coverslips, loaded with bacterial cells, and imaged using appropriate microscopy equipment.

References:

[1] R. R. Robert B. Saper, Zinc: An Essential Micronutrient. Am. Fam. Physician. 79, 768 (2009).

[2] S. Tan, D. Guschin, A. Davalos, Y.-L. Lee, A. W. Snowden, Y. Jouvenot, H. S. Zhang, K. Howes, A. R. McNamara, A. Lai, C. Ullman, L. Reynolds, M. Moore, M. Isalan, L.-P. Berg, B. Campos, H. Qi, S. K. Spratt, C. C. Case, C. O. Pabo, J. Campisi, P. D. Gregory, Zinc-finger protein-targeted gene regulation: genomewide single-gene specificity. Proc. Natl. Acad. Sci. U. S. A. 100, 11997–12002 (2003).

[3] C. Andreini, I. Bertini, in Encyclopedia of Metalloproteins (Springer, New York, NY, 2013), pp. 2549–2554.

[4] S. R. Gordon, S. Vaishnava, Zinc supplementation modulates T helper 17 cells via its effect on gut microbiome. The Journal of Immunology. 204, 83.18–83.18 (2020).

[5] O. Koren, E. Tako, Chronic Dietary Zinc Deficiency Alters Gut Microbiota Composition and Function. Proc.AMIA Annu. Fall Symp. 61, 16 (2020).

[6] F. Wu, C. Dekker, Nanofabricated structures and microfluidic devices for bacteria: from techniques to biology. Chem. Soc. Rev. 45, 268–280 (2016).

[7] D. Binder, C. Probst, A. Grünberger, F. Hilgers, A. Loeschcke, K.-E. Jaeger, D. Kohlheyer, T. Drepper, Comparative Single-Cell Analysis of Different E. coli Expression Systems during Microfluidic Cultivation. PLoS One. 11, e0160711 (2016).