Microfluidic Device for Studying Harmful Algal Blooms

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Primary CNF Tools Used: ABM contact aligner, YES polyimide bake oven, MVD100

Abstract:

Harmful algal blooms (HABs) are increasing worldwide causing degradation of lake ecosystems, and endangering recreational and drinking water resources. The exact mechanisms that lead to harmful algal blooms remain elusive, but they are often correlated with many chemical and physical environmental cues including nutrients, temperature, and fluid flows. Current assay formats such as in pond and test tubes are not appropriate for probing the role of many environmental factors. Here we developed a microfluidic platform, where cells are cultured in an array of microhabitats with controlled nutrient and chemical gradients. Using this platform, we explored the roles of cell-cell communication signals in the growth, motility and clustering of a common HAB forming cyanobacteria, *Microcystis aeruginosa*. Our ultimate goal is to find a set of environmental conditions under which rapid cyanobacteria growth is triggered and use this knowledge to discover effective control solutions for HABs.

Summary of Research:

Microfabrication of this microfluidic device is challenging since the height of the side channels and the microhabitats are different as shown in Figure 1. The double-layer SU-8 method was chosen to fabricate this device. The wafer was first dehydrated in an oven to remove water vapor. SU-8 2100 was spun on the silicon wafer at 3000 rpm for 30 seconds to form the first 100 μm thick layer. The edge bead was removed using acetone-wetted wipes. Then, a soft bake was performed on the wafer by ramping up the temperature slowly at 2°C/min from room temperature to 65°C in an hour. Then, the temperature was increased to 95°C at 2°C/min and was left on the hot plate overnight. Next day, the wafer was cooled down to room temperature slowly by turning off the hot plate.

The first layer of SU-8 was exposed to the microhabitat pattern using the 365 nm filter at 250 mJ/cm^2 on a contact aligner (ABM contact aligner, ABM, Inc., Silicon Valley, CA). Then, the second layer of SU-8 was poured and spun at 3000 rpm for 30 seconds. Edge bead removal was performed using the acetone-wetted wipes. Then, the soft bake was performed. Using 320 mJ/cm^2 on the same contact aligner using the align marks, both layers of SU-8 were exposed to the pattern of side channels. Then, a post-exposure bake was performed. For post exposure bake, the wafer was heated to 65°C from room temperature at 2°C/minutes and maintained for five minutes. Then, the wafer was ramped up to 95°C at 2°C/min and was maintained for 15 minutes. Then, the wafer was cooled down to room temperature by turning off the hotplate. Then, the wafer was developed using the SU-8 developer. Isopropanol was used to rinse the wafer. Hard bake was performed using the YES polyimide oven. Finally, FOTS treatment was done using the MVD100.

References:

[1] Beum Jun Kim, Lubna V. Richter, Nicholas Hatter, Chih-Kuan Tung, Beth A. Ahner, and Mingming Wu, An array microhabitat system for high throughput studies of microalgal growth under controlled nutrient gradients, Lab Chip, 15: 3687-3694 (2015).

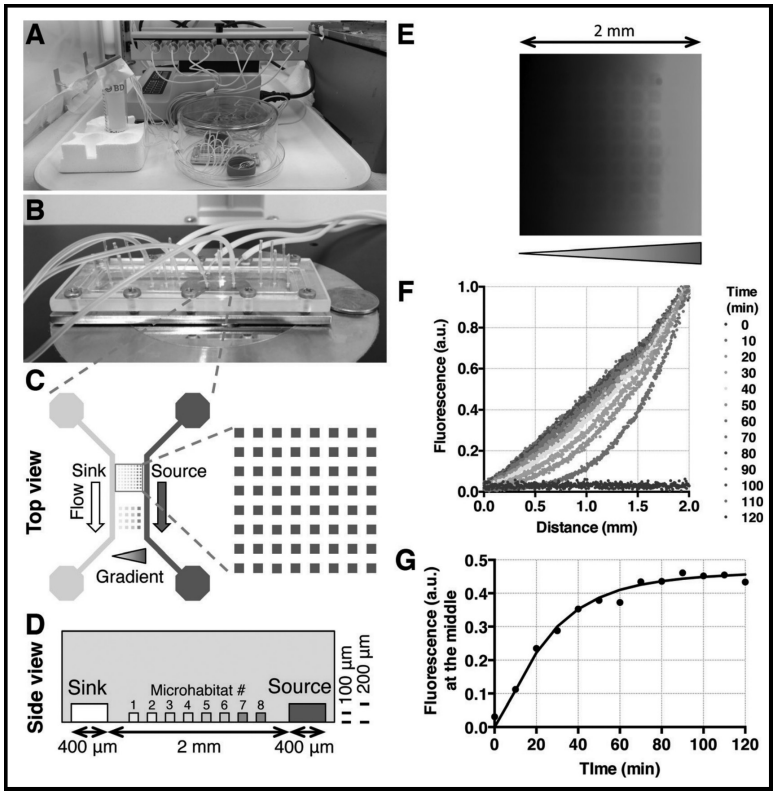

Figure 1: Microfluidic platform design and gradient characterization. A. An image of the microfluidic platform under fluorescent light for the growth of photosynthetic microalgae. The microfluidic platform is enclosed in an upside-down Pyrex® container with water reservoirs for humidity control. A syringe pump is used to perfuse media through the device. B. An image of the microfluidic platform on a microscope stage. Each platform contains four individual devices patterned in an agarose gel membrane. Tubing is used for connecting to the syringe pump for flow control. C. Top view of a device. Nutrients/buffers flow through the two side channels and form a nutrient gradient in the microhabitat array area through molecular diffusion. The top array contains 64 microhabitats, each has a dimension of 100 µm × 100 µm × 100 µm, with a gap of 100 µm between the two adjacent habitats. The bottom array contains 16 microhabitats, each has a dimension of 200 µm × 200 µm × 100 µm, with a gap of 200 µm. Cells are pre-seeded before sandwiching the agarose gel membrane between a plastic manifold and a glass slide. D. Side view of a device. The distance between the source and sink channels is 2 mm, and the cross-sectional area of each side channel is 400 µm × 200 µm. The nutrient concentration is the same for each column of the array, with the column number labeled. E. A fluorescence image of the array microhabitat taken at t = 1 h, where t = 0 is defined to be the time when fluorescein/buffer are introduced into the side channels. F. Time-evolution of fluorescence intensity profile in the array microhabitat area with a 10 min interval and total time duration of 120 min. Each colored line represents the fluorescence intensity profile at a time point. G. Experimental results (dotted line) are validated against that (solid line) from COMSOL computation. Time evolution of fluorescence intensity in the middle of the array (or 1 mm away from sink channel) is shown here.