Dual-Gradient Microhabitat Platform for Microalgae Growth

CNF Project Number: 2262-13
Principal Investigator(s): Dr. Mingming Wu
User(s): Fangchen Liu

Affiliation(s): Department of Biological and Environmental Engineering
Primary Source(s) of Research Funding: United State Department of Agriculture - National Institute of Food and Agriculture
Contact: mm272@cornell.edu, FL373@cornell.edu
Website: http://biofluidics.bee.cornell.edu/
Primary CNF Tools Used: Heidelberg mask writer DWL2000, ABM contact aligner, P10 profilometer, MVD 100

Abstract:

The occurrence of harmful algal blooms (HABs) is increasing at an alarming rate worldwide, threatening water resources and aquatic ecosystems. Nutrients are known to trigger the onset of HABs and systematic investigation at cellular level is lacking. To study the combination effects of multiple nutrients on microalgae growth in a high throughput way, a dual-gradient microhabitat platform was designed, fabricated, and characterized.

Summary of Research:

Harmful algal blooms, or HABs, are serious environmental problems, where a sudden growth of algae or cyanobacteria poses threat to freshwater and marine ecosystems. HABs deteriorate drinking water quality and have huge environmental and economical costs. Nutrient enrichment is believed to be the fundamental cause of HABs, and climate change may further intensity the problem [1]. However, there lacks a quantitative/mechanistic understanding of the roles of environmental factors in the onset of HABs at cellular level. The goal of this project is to investigate the synergistic roles of multiple environmental factors in the growth of cyanobacteria.

Environmental conditions known to affect algae growth include nutrients, mainly nitrogen (N) and phosphorous (P), light intensity and temperature. These conditions are hard to control in nature, and also cannot be quantified in a high throughput way in flasks and chemostats. Previously, an high throughput array microhabitat platform has been developed in our lab that is suitable for monitoring growth of photosynthetic microbes [2]. This platform is capable of generating a stable single nutrient gradient. Using this platform, we discovered that the growth rates of *Chlamydomonas reinhardtii* in the presence of NH_4Cl gradient fit into a modified Monod kinetics model with the half saturation constant of NH_4Cl to be 1.2 ± 0.3 μM.

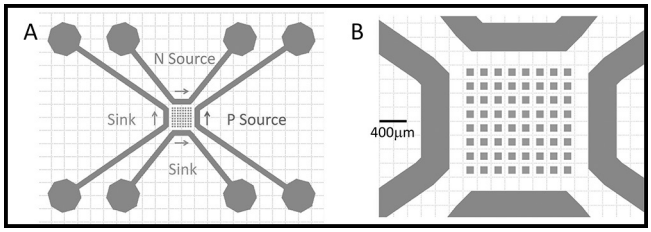

Figure 1: Dual-gradient microfluidic platform design. A. Top view of a device. B. A zoomed-in view of microhabitats and channel. The 8 × 8 array of 100 μm cubic habitats are separated by 100 μm from each other. These habitats are surrounded by four channels with width of 400 μm and height of 200 μm. N source and P source runs through the top and right channel respectively, and the other channels are sink channels. A gradient is generated for each chemical species in the microhabitat array region through molecular diffusion.

In this project, we developed a microhabitat platform that can provide dual nutrient gradients to facilitate a more realistic condition found in nature. The design of our device is shown in Figure 1, which consists of 64 microhabitats in the form of an 8 × 8 array and each habitat is 100 μm × 100 μm × 100 μm. The microhabitat array is surrounded by two sets of side channels each with the width of 400 μm and height of 200 μm. In each set of side channels, we can run source media (with N, or P) and blank media respectively, and a stable gradient can be simultaneously generated along vertical and horizontal directions.

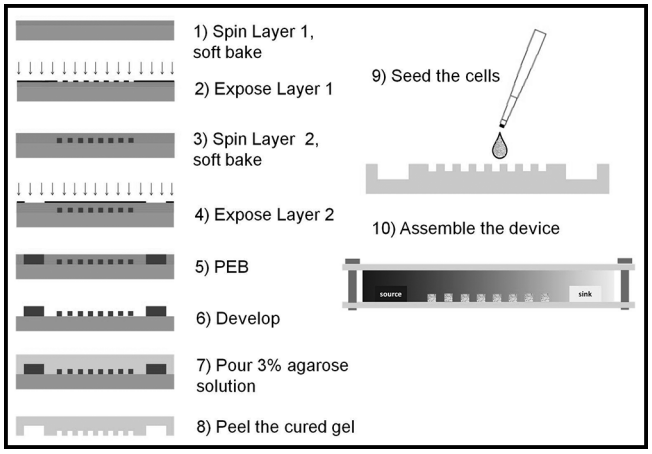

Figure 2: Schematics of a two-layer SU-8 photolithography procedure and the final microfluidic device assembly. First, a 100 µm resist layer was spun on wafer, soft baked and exposed. Then, another 100 µm layer was spun on top and baked together overnight, followed by the second exposure and post exposure bake (PEB) for the 200 µm structures. The unexposed resist was then developed and the structures went through hard bake. For device assembly, the pattern was imprinted on an agarose gel, and cells were seeded in the microhabitats. The gel was then sandwiched between glass slide and manifolds and tightened by screws.

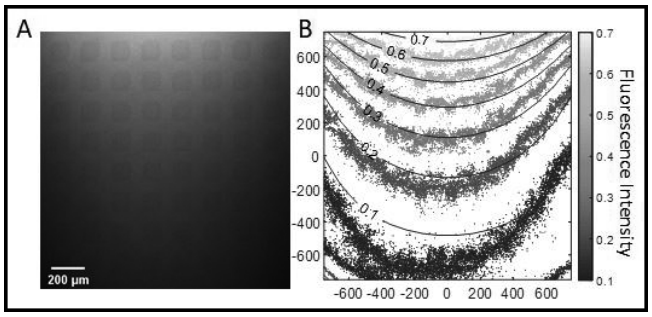

Figure 3: Characterization of the device using fluorescein (FITC) dye. A. FITC solution was introduced to top channel and buffer solution without FITC were introduced in the other three channels at t = 0. The fluorescence image is taken at the middle where the microhabitats are at t = 60 min. B: Contour plot of simulated (lines) and experimental (dots) concentration fields at t = 60 min. Concentration is scaled such that the concentration in source channel is 1 and sink channel 0.

Soft lithography was used to make this dual-gradient microhabitat platform, which involves fabricating the silicon master mold and molding the pattern onto agarose gel for device assembly. Schematics of the step by step procedure are shown in Figure 2. The silicon master mold was fabricated using two layer SU-8 negative resist photolithography, since the channels are 200 µm high and the microhabitats are 100 µm high. The post exposure bake (PEB) of the first layer of photo resist was combined with the soft bake of the second layer of the photo resist. Also, it was found that slow temperature ramping and relaxation time after each bake is critical to minimize internal stress in order to prevent resist detachment problem. After developing, the height of the feature were measured using P10 profilometer and a layer of FOTS was deposited using molecular vapor deposition (MVD100) to increase the surface hydrophobicity for easier demolding of agarose gel. To transfer the pattern, boiled 3% agarose solution was poured on the silicon master and peeled once it cured.

The gradient behavior of this dual-gradient platform was characterized using Fluorescein (FITC) dye (Figure 3). 50 µM FITC was flown through the top channel, and blank media in the other channels at t = 0, and the stable gradient was established via molecular diffusion through the agarose gel. A fluorescence image at 60 min was plotted together with results from a 2D COMSOL simulation, which uses Fick's second law for diffusion and fixed concentrations at the channels as boundary conditions. In Figure 4B, the experimental field matches the simulated concentration field, which indicates the establishment of both gradients. Currently, microalgal growth in both nitrogen and phosphorous gradient are being studied at the same time in this platform.

References:

[1] Paerl, Hans W., et al. Environmental Science and Technology (2018): 5519-5529.

[2] Kim, Beum Jun, et al. Lab on a Chip 15.18(2015): 3687-3694.