Dual-Gradient Microhabitat Platform for Microalgae Growth

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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 a 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. Using the platform, the synergistic effect of nitrogen and phosphorous on the growth of model microalgal *Chlamydomonas reinhardtii* was revealed.

Summary of Research:

Harmful algal blooms, or HABs, are serious environmental problems, where a sudden growth of algae or cyanobacteria poses a 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 a 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, a high throughput array microhabitat platform has been developed in our lab that is suitable for monitoring growth of photosynthetic microbes [2], which is capable of generating a stable single nutrient gradient. Using this platform, we discovered that the growth rates of *Chlamydomonas reinhardtii* (*C. 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.

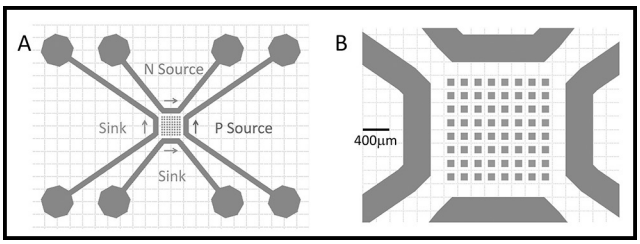

Figure 1: Dual-gradient microfluidic platform design. A. Top view of a device. B. A zoomed-in view of microhabitats and channel. The 8 × 8 array of 100 μm cubic habitats are separated by 100 μm from each other. These habitats are surrounded by four channels with width of 400 μm and height of 200 μm. 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.

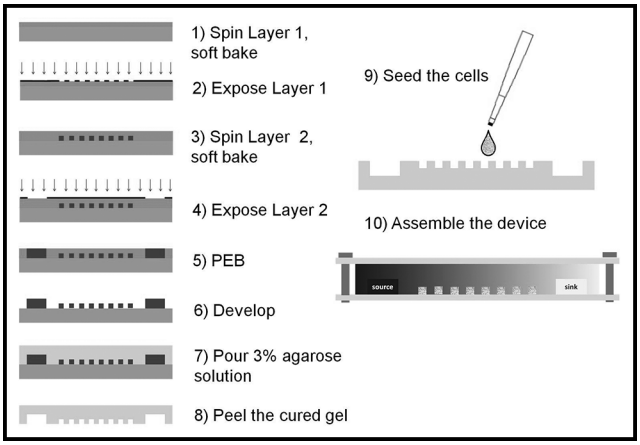

Figure 2: Schematics of a two-layer SU-8 photolithography procedure and the final microfluidic device assembly. First, a 100 μm resist layer was spun on a wafer, soft baked and exposed. Then, another 100 μm layer was spun on top and baked together overnight, followed by the second exposure and post exposure 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.

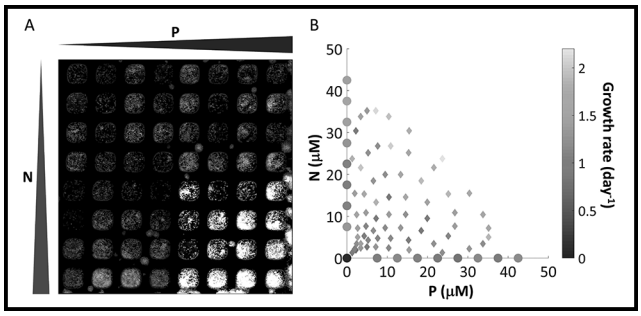

Figure 3: Growth of C. reinhardtii *under nutrients (N, P) gradient. A. Fluorescence images of nutrients co-limited cells growing under N and P dual gradients at day 4. B. The growth rate of* C. reinhardtii *under: control condition, no N or P (dot at the origin), single P gradient (dots on* x *axis), single N gradient (dots on* y *axis), and dual-gradient (all the diamonds). Shade is coded for the value of the growth rate.*

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 was measured using the P10 profilometer, and a layer of FOTS was deposited using molecular vapor deposition (MVD 100) 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 fluorescent dyes (for details see reference [3]).

The platform was used to study the growth of *C. reinhardtii* under nitrogen (N) and phosphorous (P) gradients. Experiments with N, P co-limited cells showed that N and P synergistically promoted cell growth (Figure 3A), while no discernible response was observed when single N or P gradient was imposed [3]. Growth rates under single gradient, dual-gradients, and control conditions were obtained and organized in Figure 3B, which could benefit the quantitative study of microalgal growth. These results proved the enabling power of the dual-gradient microhabitat platform in screening effects of multiple environmental factors in photosynthetic cell growth [3].

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.

[3] Liu, Fangchen, et al. Lab on a Chip 20.4 (2020): 798-805.