ACTIVE MODULATION OF SYNTHETIC MICROBIAL SIGNALING PATHWAYS USING MICROFLUIDIC CHEMICAL INTERFACES

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ABSTRACT

This work presents microfluidic chemical interface systems enabling the active modulation of synthetic microbial signaling pathways by controlling the diffusion and reaction of cell-signaling molecules by directly activating and inactivating gene expression in an initially homogenous population of cells. The strategy is to combine a microfluidic system that acts as a distributed inhibitor system and a synthetic gene circuit that contains a positive feedback loop activated by acyl-homoserine lactone (AHL) to form synthetic microbial patterns; the activator molecules are AHL and the inhibitors are hydroxyl ions.

KEYWORDS: Synthetic Biology, Microfluidics, *E. coli*, Pattern Formation

INTRODUCTION

Many biological patterns observed in nature are mathematically described by two-component Turing's reaction-diffusion system [1]. The classical Turing system requires an inhibitor molecule which diffuses much faster than the activator molecule; this condition has made it difficult to construct a synthetic Turing system in cell culture. In this work, microfluidic chemical interface systems are employed to demonstrate a hybrid, synthetic Turing reaction-diffusion system [2-4].

EXPERIMENTAL

As illustrated in Fig. 1, the microfluidic pH gradient generation system was used to form a linear pattern into a monolayer of bacterial cells. A pH-sensitive cell-signaling pathway along with a GFP reporter was constructed on a plasmid and transformed into *E. coli*. AHL is produced by LuxI and then AHL activates the pLux promoter to create a positive feedback loop. *E. coli* cells were grown on top of the agar layer and a pH gradient was produced across the agar by making two different pH media at both sides; AHL was mixed with neutral pH media but it degrades at high pH (at pH=9 or above, $\tau_{1/2}$=~20min). As a control, the same base strain of *E. coli* cells were transformed with a plasmid which constitutively expresses RFP and were used to account for growth inhibition by high pH. Cells were grown overnight before fluorescence images were taken and the images were analyzed using Image J. GFP/RFP microscopy images were stitched together to quantify the fluorescent signals.

RESULTS AND DISCUSSION

As seen in Fig. 1 (left), GFP signals significantly decrease along the pH

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gradient from pH=5.3 to pH=9.3 while RFP signals are almost uniform. This is because the LuxI-GFP feedback loop is attenuated by the inhibitor (hydroxyl ions) because AHL degrades at high pH. As a control, when the same experiment was repeated in the absence of a pH gradient, no difference of fluorescent signals (distinct pattern of GFP) was observed. As seen in the graph, GFP signals match with the qualitative results and RFP signals obtained from co-cultured cells with LuxI-GFP cells support that the pH gradient significantly affects pattern formation but not much in cell growth.

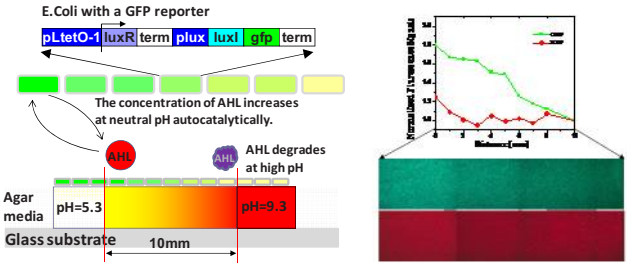

Figure 1. Schematic of a pH sensitive cell-signaling pathway with a GFP reporter built into E. coli and a microfluidic device generating a pH gradient into a monolayer bacterial cell. The spatial pattern formation by gene expression is actively controlled by the pH gradient that modulates the degradation of AHL. Constitutively expressed RFP was used to find out effects of pH's on cell growth.

To verify the experimental results in Fig. 1, the cells were tested in different pH media using a 96-well plate and a microplate reader. As shown in Fig. 2, LuxI-GFP cells expressed a smaller amount of GFP at pH of 8 or above than neutral pH's which was quantified by measuring fluorescence signals excitated at blue light (~475 nm). As a control, the same strain cells engineered to constitutively express GFP at the same pH condition were also tested, but the variation of fluorescence signals of GFP was much smaller than the LuxI-GFP; about 30 AU (arbitrary unit) compared to 250 AU which is approximated by subtracting min. value of 250 at pH=9.02 from max. value of 350 at pH 7.2 at 14 hours.

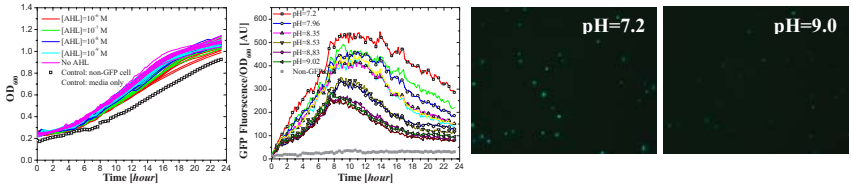

Figure 2.Characterization of the LuxI-GFP operon using a microplate reader (Tecan, Safire) shows that pH of 8 or above degrades AHL. Open shapes are at 12 hours (saturation) and filled shapes are at 6 hours (exponential growth).

As another control, the strain cells containing only LuxI construct without GFP and the LuxI-GFP in the absence of AHL were tested and the GFP signals of both of them were found significantly low. Given that the cell division is interfered with ex-

tracellular pH's, average GFP signals were derived by dividing the accumulated GFP signals by OD_{600} and the result turned out that the LuxI-GFP cells response to external pH values as designed, showing a good agreement with the results obtained using a microfluidic device.

An alternative way to produce a pH gradient is to use electrolysis of water. This method can generate more complex pH gradients with high resolution by using an array of microfabricated electrodes; anode generates oxygen and proton (H^+) while cathode generates hydrogen and hydroxyl ions. In addition, the applied current can be easily altered to change the slope of a pH gradient as well as dynamically control the gradient with time. However, as seen in Figure 3, this method holds a side effect that produces chlorine; chlorine is a well known gas that kills live cells. Even though the pH gradient ranges from pH=5.5 (anode) to pH=9.5 (cathode), since the number of cells decreases along the pH gradient, fluorescence signals increase from left to right, which is opposite to the results shown in Fig. 1 and Fig. 2. When no current was applied to, GFP signals showed no differences from the anode to the cathode.

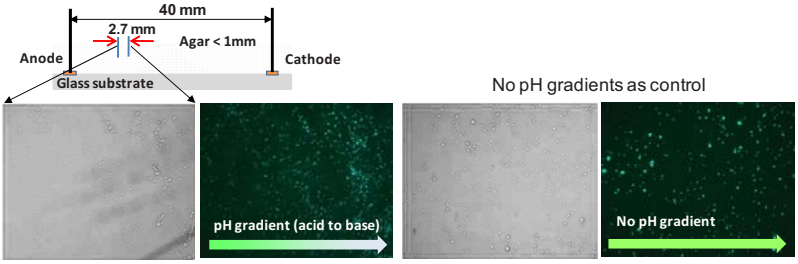

Figure 3. Microfabricated electrodes were used to produce pH gradients. Due to chlorine generated during the electrolysis, the number of cells decreases from the anode to cathode, showing opposite GFP signals along the pH gradient.

CONCLUSIONS

Microfluidic chemical interface systems that can form synthetic microbial patterns by modulating a cell signalling pathway were demonstrated and compared with each other. The approaches shown in this work hold a high potential for better understanding Turing's model. Lastly, it is hoped that this work helps to develop multicellular organisms by actively modulating signaling pathways of developing cells.

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