Developing a Novel Microfluidic Device for the Study of Molecular Communication Between Bacterial Colonies

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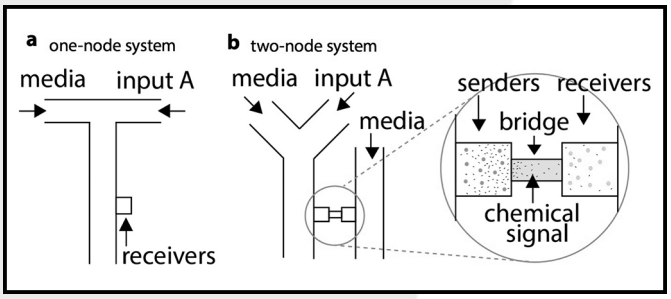


Figure 1: The concept design developed from our current one-node design into our two-node system.

Introduction:

Understanding the molecular communication between bacteria is a key component in building biosensors that utilize genetically modified bacteria for applications such as environmental monitoring. Currently, we have the capacity to study one receptor bacterial colony's response to an artificial chemical signal (i.e., a one-node system) via a microfluidic device; however, studying the communication between two bacterial colonies (i.e., a two-node system) is key to improving the robustness of biosensors. Previous studies have created devices that can pass a gaseous signal between isolated colonies [1], but devices that study aqueous signals have cross-contamination issues [2], or limited versatility of signal input [3].

This project focused on the creation of the first microfluidic device that allows for the study of various communication schemes (e.g., pulses, step functions) via an aqueous signal between sending and receiving bacterial colonies in one microfluidic chip. Our design, seen in Figure 1, is based on isolating sending and receiving colonies of bacteria in separate chambers and connecting them via a bridge that contains a porous polymer monolith that acts as a filter. Patterning the polymer monolith posed a key challenge. The chemical signal diffuses from the senders through the bridge to the receivers. However, the chemical signal doesn't entirely diffuse across the bridge, so we manipulated the geometry of our device to control the signal loss.

Experimental Methods:

Polydimethylsiloxane (PDMS) devices were fabricated using a 65g total 10:1 ratio of polymer to crosslinker (Silgard 184 Elastomer) poured onto a silanized SU-8 master mold and cured for four hours minimum at 60°C.

The device was masked off to photopattern the monolith. In fabricating the porous polymer monolith, the PDMS channels were first surface treated. A 0.25M 2,2'-dimethoxy-2-phenylacetophenone (DMPAP) in acetone solution rinse was flooded through, and then a monomer solution of a 1:1 ratio of methylmethacrylate and ethylene diacrylate was loaded in and UV exposed for 40s with an 8W of 365 nm UV. This was then flushed out and then the monolith solution—consisting of (by weight) 60% 1:1 methanol to 2-propanol solution (porogen), 20% butyl methacrylate (monomer), 20% ethylene dimethacrylate (crosslinker), and 0.4% DMPAP (photo initiator)—was flooded in and treated with 8W of 365 nm UV for 45 minutes.

Confocal microscopy of the fabricated monolith was performed with a Zeiss 510 laser scanning microscope. To test the blocking ability of the monolith, genetically engineered *Escherichia coli* (*E. coli*) that continually produced green fluorescent protein (GFP) was flown into the device against the monolith.

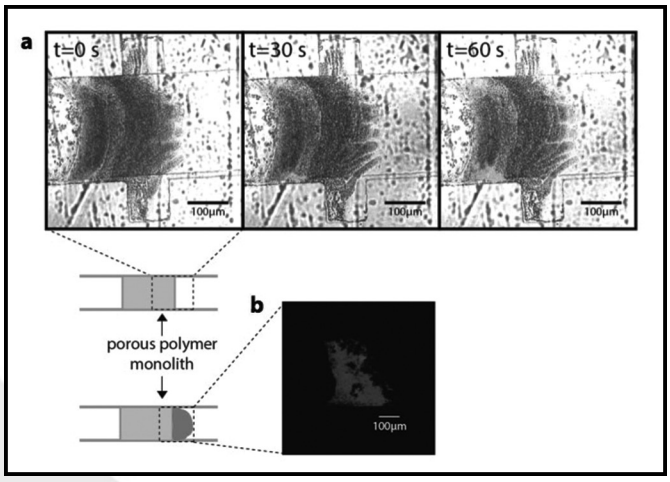


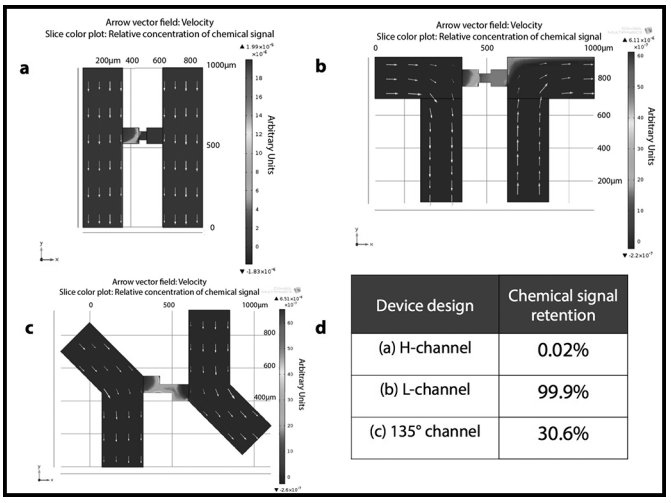
Figure 2: Microscopy images of the photopatterned monolith fabricated in an arbitrary device. (See in full color on page xxxvi.)

COMSOL Multiphysics 4.4 software was used to model theoretical chemical signal retention between the chambers in different geometries, using empirically realistic values and assuming a no flow condition in the bridge to account for biofilm buildup.

Results and Discussion:

Figure 2 depicts the confocal microscopy images of our resulting monolith. After flowing in *E. coli* that continually produced GFP, we found that the bacteria were capable of penetrating the beginning of the filter, but eventually were trapped and collect in patches, as seen in Figure 2a. In another trial of the fabrication, we imaged the bacterial plug that collected against the fabricated filter. These preliminary results show that the filter seems to be capable of preventing cross-contamination of bacterial colonies.

To manage loss of chemical signal, we engineered the device geometry to lower the velocity of the flow adjacent to the bacteria chambers by varying the channel angles. Key factors that need to be considered include the tendency for bacteria to stick to and colonize sharp turns, corners, and areas of low velocity, potentially clogging the device.



Device design	Chemical signal retention
(a) H-channel	0.02%
(b) L-channel	99.9%
(c) 135° channel	30.6%

Figure 3: COMSOL plots analyzing the chemical signal retention of varying geometries.

Figure 3 shows the COMSOL plots showing the velocity vector field and concentration gradients of varying geometries. The H-channel design retained almost no signal, given the uniformity of velocity flow adjacent to the chamber. The L-channel design minimized loss of signal, but had many sharp angles areas of low flow in the corners such that clogging would occur. The 135° angled channel design allowed for a compromise between signal retention and uncontained bacterial growth. The tunable nature of the geometry offered the full range of retention percentages. We moved forward with the 135° angled channel design, because the theoretical retention of 30.6% of the chemical signal seemed sufficient.

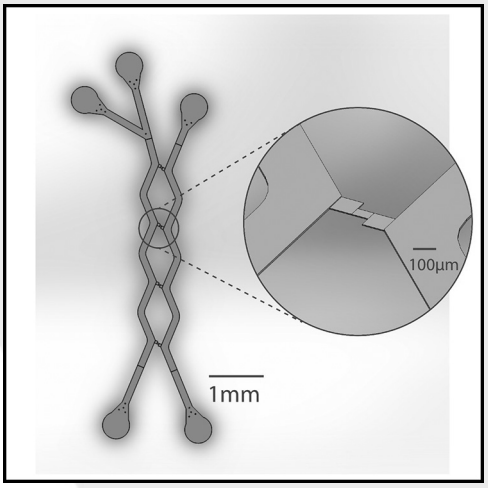


Figure 4: The final design with four chamber-pair iterations and rounded corners.

If this proves to be insufficient, one can tune the angle of the channel in the design for signal retention. Our final design is seen in Figure 4; corners are rounded to prevent bacterial adhesion.

Future Work:

Our next step is to fabricate the porous polymer monolith in the exact bridge region of our new device. We need to run experiments to see if the filter pore size provides sufficient isolation of the colonies over the time period of an experiment (~ 2 days). If insufficient, we need to adjust the recipe for the monolith to tune the pore size such that the bacteria cannot migrate through. Additionally, we need to prove that the chemical signal can freely diffuse through the monolith. With this, we will have successfully created the first microfluidic device to study an aqueous signal between two bacterial colonies.

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References:

[1] A. Prindle, et al., A sensing array of radically coupled genetic 'biopixels', Nature 481, 39-44 (2012).

[2] S. Park, et al., Microfabricated ratchet structure integrated concentrator arrays for synthetic bacterial cell-to-cell communication assays, Lab Chip 12 3914-3922 (2012).

[3] K. Nagy, et al., Interaction of Bacterial Populations in Coupled Microchambers, Chem. Biochem. Eng.Q., 28 (2) 225-231 (2014).