A PDMS-SEALED HYDROGEL DEVICE FOR RAPID AND ACCURATE GENERATION OF VARIOUS CONCENTRATION GRADIENTS

Minseok Kim, Mingjie Jia, and Taesung Kim
Ulsan National Institute of Science and Technology (UNIST), Republic of Korea

ABSTRACT
We report a microfluidic device that can rapidly and accurately generate various concentration gradients for the chemotaxis study of bacterial cells by integrating hydrogel into polydimethylsiloxane (PDMS) microchannels. We theoretically and experimentally demonstrated that the PDMS-sealed hydrogel (PSH) device produces various profiles of concentration gradients without flow-induced shear stresses. Furthermore, the device exhibits remarkable advantages over conventional hydrogel-based (CHB) devices. For example, the PSH device prevents dehydration of hydrogel; guides diffusion of chemicals; generates concentration gradients rapidly; exhibits long-term durability; and is easy to handle. Using the PSH device, we demonstrate chemotaxis responses of bacterial cells in various concentration profiles.

KEYWORDS: Chemotaxis, Hydrogel, Polydimethylsiloxane, Bacteria

INTRODUCTION
Microfluidic technologies provide many unprecedented experimental tools for bacterial chemotaxis that exhibits how cells and microorganisms sense and respond to concentration gradients of chemical environments [1, 2]. The diffusion driven CHB device has been preferred to minimize flow-induced shear stress and demonstrated stable formation of not only linear but also nonlinear concentration gradients. However, hydrogels are amorphous, soft materials that mostly consist of water (~99%), hydrogels easily become dehydrated, and the transport of molecules through hydrogels is not well guided in a desired direction. In this work, we solved the critical problems in CHB devices by sealing hydrogel with PDMS microchannel.

THEORY
The transport of small molecules in conventional diffusion-based microfluidic devices can be governed by the transient diffusion equation which is typically simplified as a complementary error function for a transient 1-D model as follows [1].

\[ c(x, t) = c_0 \text{erfc} \left( \frac{x}{2\sqrt{Dt}} \right) \]

where \( c \) represents the concentration of the chemoattractant, and \( D \) represents the diffusivity of the chemoattractant in the hydrogel or buffer solution. Therefore, the concentration gradients are theoretically asymptotic with time, and the gradients of small molecules gradually become linear when the boundary conditions are maintained (i.e., source: \( c(x = 0, t) = c_0 \) and sink: \( c(x = L, t) = 0 \)) without any convection flows between source and sink channels.

EXPERIMENTAL
As shown in Fig. 1, the microfluidic channel network mainly consisted of three parts: chemical-loading (i.e., source and sink) channels, a cell-loading (i.e., test) channel, and hydrogel-loading channels. The hydrogel-filling channel was designed to be shallow (15 µm) while the other channels (including the test channel) were designed to be deep (30 µm), as described in Fig. 1B, in order to selectively fill the hydrogel layer with a hydrogel precursor solution [3]. After curing the hydrogels, we loaded and continuously flushed the source and sink channels with a buffer solution that contains constant concentrations of chemoattractant. The cell-loading channel was designed as a test channel in which various linear and nonlinear concentration profiles could be generated. The fluid flow was stopped after the cell-loading channel was filled with bacterial cells, and a stationary state was maintained to completely eliminate any effects of convection flow on the generated concentration profiles and the chemotaxis of the bacterial cells.

![Figure 1](image-url)

**Figure 1:** A. Schematics of the PSH device. B. A cross-sectional view of the microchannel along the a-a’ C. Constructed hydrogels in PDMS microchannels and an experimental set-up for chemical boundary conditions.
RESULTS AND DISCUSSION

To quantitatively characterize and compare a PSH device (Fig. 2A) and a CHB device (Fig. 2B), we fixed the source-, sink-, and test-channel geometry \( (h_c = 30 \, \text{m deep}, \, w_c = 300 \, \text{m wide}) \) and the hydrogel diffusion layer \( (h_h = 15 \, \text{m deep and } \, w_h = 500 \, \text{m wide}) \). The source channel (left) was filled with a chemoattractant solution (\( \alpha \)-methyl aspartate 1 mM, \( D = 1.4 \times 10^{-9} \text{ m}^2/\text{s} \) in water), while the sink (right) channel was only filled with water. The same diffusivity, which was assumed to be uniform across the middle chamber, was used for both models. The PSH model reached a steady state in 10 min because of the thin, confined PDMS geometry while the CHB model took several hours to reach a steady state. As expected, the chemoattractant molecules diffused much faster in the PSH device than they did in the CHB device. This finding means that our device can be used to rapidly generate accurate linear concentration gradients in the test channel across the hydrogel layer.

We used a 50 \( \mu \text{M} \) FITC solution to characterize a PSH device. As shown in Fig. 3A, the dyes diffused from the source to the sink channel across the test channel, which was filled with only a buffer solution, at the center of the device. Fig. 3B shows the normalized intensities of the fluorescence along the \( x \)-axis from the source channel across the hydrogel layer to the sink channel. The intensities of the fluorescence show an almost linear concentration gradient regardless of the position along the \( y \)-axis, which is consistent with each other. Furthermore, the concentration gradient was measured daily for a week, demonstrating the long-term durability and stability of the linear concentration gradient (Fig 3C). The nonlinear concentration gradients are generated along the various profiles of the test-channels. As shown in Fig 3D, the linear and nonlinear profiles of the concentration gradient of the chemoattractant in the test channel in the presence of global constant source and sink channels. The quantitative result (Fig 3E) demonstrates the concentration profiles depend on the shape of the pre-defined test channel by the hydrogel layer.

![Figure 2: A and B. Numerical simulation of the diffusion in PSH device and CHB device, C. Quantitative result of the simulation that indicate the adapting times of the devices to a chemical environment.](image)

![Figure 3: A and B. Generation of a steady and linear concentration gradient between source and sink channels. C. Long-term durability of the stable concentration gradient, D and E. Formation of nonlinear concentration gradients arbitral profiles of test-channels pre-defined by hydrogels.](image)
We used a PSH device to study the chemotaxis of Escherichia coli (E. coli). We fabricated a straight test channel in the middle of the hydrogel layer, as shown in Fig. 4A. We loaded a 100 μM aspartate solution as a chemoattractant into the source channel and only a buffer solution into the sink channel. We photographed the intensities of the fluorescence emitted from E. coli cells and measured the biased chemotactic responses of the cells after 10 min; most of the E. coli migrated toward the high concentration of chemoattractants in the linear concentration gradient.

We loaded E. coli cells in the same manner into the curvilinear test-channels and then completely stopped the convection flows to observe the chemotactic responses of the cells (Fig 4B and 4C). The different chemotactic responses of the E. coli are observed along the concave and convex channels because the local concentration gradient varies along the channels. For example, the cells showed stronger positive chemotactic responses in the steep concentration gradient (|Δc/ΔS1|) than in the shelvy one (|Δc/ΔS2|). This is why more cells are observed in the concave channel than the in the convex one in the upper side of the hydrogel layer even though the global concentration of the chemoattractant remains constant.

CONCLUSION
We developed the PSH device to generate various concentration gradients for the chemotaxis study of bacterial cells. Since the combination of PDMS and hydrogel complements the limitations of conventional homogeneous material-based device, we believe that the device can be widely applied to not only chemotaxis assays for motile microorganisms and mammalian cells but also to the study of cell responses in diverse chemical environments.

ACKNOWLEDGEMENTS
This work was supported by a grant from the Next-Generation BioGreen 21 program (SSAC, o. PJ00954905), Rural Development Administration, Republic of Korea and by the National Research Foundation of Korea (RF) grant funded by the MEST (RF-2009-C1AAA001-2009-0093499).

REFERENCES

CONTACT
*T. Kim, tel: +82-52-217-2313; tskim@unist.ac.kr