Generation of Stable Complex Gradients Across Two-Dimensional Surfaces and Three-Dimensional Gels

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Many chemical and biological processes are dependent on molecular gradients. We describe a new microfluidic approach that can be used to produce spatiotemporal gradients across two-dimensional surfaces and three-dimensional gels under flow-free conditions. Free diffusion between dynamically replenished flow channels acting as a sink and source is utilized to give rise to stable steady-state gradient profiles. The gradient profile is dictated by the engineered design of the device's gradient-generating region. Different designs can yield both linear and non-linear gradients of varying profiles. More complex gradients can be made by juxtaposing different designs within a single gradient-generating region. By fabricating an array of designs along the gradient-generating region, different gradient profiles can be generated simultaneously, allowing for parallel analysis. Additionally, simple methods of localizing gels into microdevices are demonstrated. The device was characterized by experimentally obtained gradient profiles of fluorescent molecules that corroborated closely with a simulated finite element model.

A wide range of chemical and biological phenomena are influenced by gradients of molecular species.[1−4] Recent progress in microfabrication and microfluidics has resulted in innovative approaches to generate and maintain spatial and temporal gradients.[5−9] Two general approaches have been described: (1) forming gradients perpendicular to continuously flowing streams of varying concentration and (2) forming gradients along a channel by free-diffusion between a source and a sink. The first method is advantageous for producing stable complex gradients,[10] but the constant flow and shear stress are not compatible with experiments such as crystallization and chemotaxis of non-adherent or weakly adherent cells (i.e., bacterial and yeast). Although the constant flow can maintain uniform growth factor conditions, it can also introduce unwanted perturbations to autocrine/paracrine factors for cell signaling investigations. To overcome some of these drawbacks, several approaches have utilized free diffusion to produce gradients in static environments.[5−9] However, because they rely on free diffusion, the gradient profile changes with time, and it is difficult to obtain a steady-state gradient over long periods. In addition, the shape of the gradient profiles that can be generated is limited, and it has not been possible to generate gradients across three-dimensional (3D) environments.

This paper describes the design and characterization of a monolithic microfluidics-based device that can generate an array of complex steady-state soluble molecular gradients in flow-free two-dimensional (2D) and 3D environments. 3D hydrogels are increasingly used in the investigation of many cell behaviors[11,12] as they simulate in vivo conditions better than 2D models. Investigations on the invasive migration of metastatic cancer cells and stem cell niches can benefit greatly if complex, stable molecular gradients can be achieved across 3D gels in flow-free conditions. Figure 1A,B shows two examples of the device, fabricated in poly(dimethylsiloxane) (PDMS)[13] that generates gradients across 2D surfaces and 3D gels, respectively. The design consists of two distinct regions: (1) two parallel main channels that act as the source and sink that are continuously replenished by flow and (2) a gradient-generating region (GGR) located between the source and sink channels. This principle can be applied to build more complex profiles by simply engineering the shape of the GGR. Continuously replenished source and sink are advantageous over static reservoirs because their concentration can be kept constant, and thus the gradients can be maintained at a constant profile in steady-state.

The device generates gradients across the GGR by free-diffusion between the source and sink main channels. For gradients over 2D surfaces, convective flow into the GGR is minimized by designing the height of the GGR (3 μm) to be significantly smaller than that of the main channels (100 μm). The large difference in GGR channel height yields an even larger difference in fluidic resistance, causing flow to take the path of least resistance (main channel) rather than entering the GGR, allowing only diffusive transport. Additionally, post structures can be fabricated along the interface of the GGR and main channels to provide additional resistance when large openings are needed

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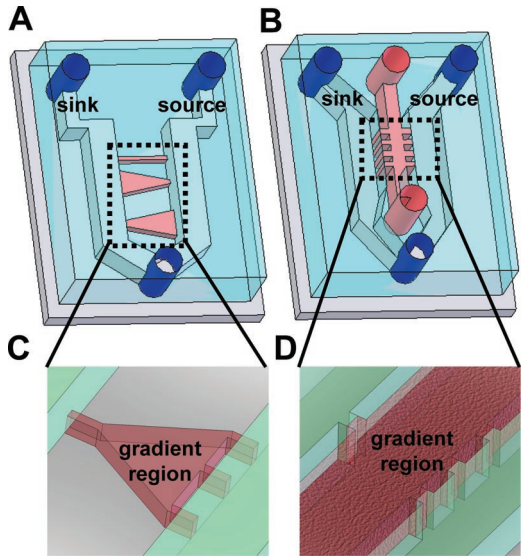

Figure 1. (A,B) Schematic of PDMS device with red region designating GGR and blue highlighting flow inlets and outlet: (A) GGR is a set of microchambers designed for gradients across a 2D surface; (B) GGR is a continuous channel with posts at the interface with main channels for gradients across a 3D gel. (C) Close-up of microchamber in a 2D surface gradient with posts at the interface of the GGR and the tapered microchamber to produce a nonlinear gradient. (D) Close-up of GGR for a 3D gel gradient with asymmetric posts designed to produce a nonlinear gradient.

for nonlinear gradient profiles, as shown in Figure 1C. A similar approach can be used to generate gradients across 3D gels, as shown in Figure 1D (see Supporting Information for photomicrograph of selectively filled GGR). This approach allows equal heights of the main channel and GGR by selectively filling the GGR with a 3D matrix (i.e., collagen gel), which increases the fluidic resistance, minimizing flow penetration into the GGR region. Additionally, the gel can also facilitate the 3D environments for cell biology applications.

Linear gradients are generated when the boundary conditions are such that the molecular flux area is constant across the GGR, as in a straight channel.[5,9] In order to generate nonlinear profiles that are more relevant for both chemical and biological conditions,[14] the flux area needs to differ across the GGR. This was achieved by two different methods, which can be used for either 2D surfaces or 3D gels: (1) an array of discrete microchambers (Figure 1A) and (2) a continuous channel with openings at the interface of the GGR (Figure 1B) to the source and sink channels. For the first method, the gradient profile of each microchamber is determined by two factors: the geometric design of the microchamber and the relative openings to the source and sink. Linear profiles are produced for a straight channel, where slope depends on the length of the GGR. Nonlinear profiles are produced in an asymmetric design by tapering the microchamber, which causes a continual imbalance of the in-flux and out-flux area between the source and sink channels through which molecules diffuse. The degree of nonlinearity can also be changed (albeit slightly) by curved microchambers as compared to the straight tapering shown in Figure 1C. The second factor, which is relevant for both methods, is the ratio of the number of sink to source openings (area of GGR through which molecules diffuse). Linear gradients are produced when the openings are symmetric and evenly distributed at opposite ends since the in-flux and out-flux areas of the diffusing molecules are equal.

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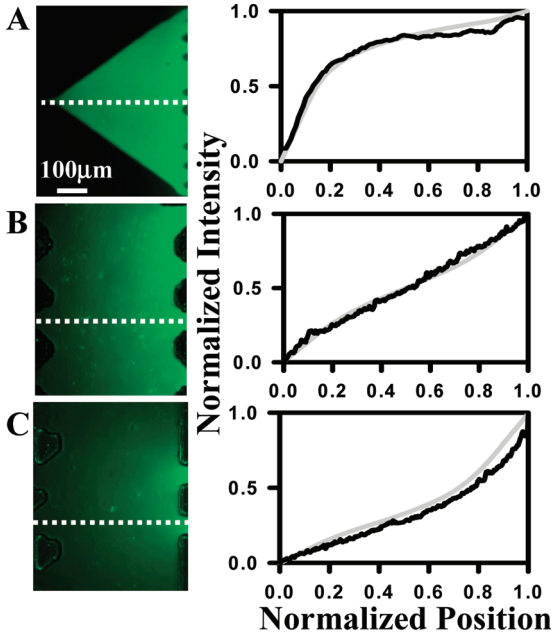

Figure 2. Fluorescent images and corresponding experimental (black) and theoretical (gray) gradient profiles across a GGR; intensity profiles taken across the white dash line. (A) 2D chamber shown in Figure 1A was used to produce a concave down nonlinear profile. The microchamber was 400 μm wide with openings 30 μm wide. (B) Gradients across 3D Matrigel were produced using the design shown in Figure 1B. Linear profiles were produced by designing the same number of openings to source and sink channels. (C) Mismatched number of openings to source (2) and sink (5) channels, resulting in a nonlinear profile. The openings were 50 μm wide.

Nonlinear gradients are produced when the number of openings are unbalanced, causing an unequal in-flux and out-flux area in the GGR. The degree of nonlinearity can be enhanced by increasing the ratio of openings and shortening the width of the GGR. For both methods, the gradient range is controlled by the concentrations of the source and sink, which can be further controlled temporally by switching the inlet solutions.

More complex gradient profiles can be made by a serial combination of different microchamber designs within one GGR. For example, juxtaposing a concave down and concave up nonlinear profile will yield a sigmoidal gradient profile (see Supporting Information). For the first method, this is achieved by having a "bow-tie"-shaped microchamber with wide openings to the source and sink channels that tapers toward the middle. For the second method, this is achieved by the same principle where two GGR regions have many openings to the source and sink channels on the outer sides but few connecting openings in the middle. Additionally, since various types of microchamber and post designs can be integrated during fabrication (see Supporting Information), a variety of both linear and nonlinear profiles can be generated on a single device for high-throughput experiments.

Shown in Figure 2 are three fluorescent micrographs and their corresponding experimental (black) and theoretical (gray) gradient profiles. The theoretical data was generated by a finite element modeling program under diffusion (see Supporting Information). The fluorescence gradient profiles were generated by infusing fluorescein into the right main channel (source) and deionized water into the left main channel (sink). Figure 2A is a concave down nonlinear gradient across a 400 μm tapering microchamber generated by having a large number of openings (11) to the source channel and a small number of openings (1) to the sink channel. Figure 2B,C shows gradients generated across a 3D

Matrigel. Figure 2B has symmetric and evenly distributed post structures, as in Figure 1B, yielding a linear gradient. Figure 2C has a mismatched number of openings (2 source and 5 sink), producing a nonlinear profile. Steady-state gradient profiles are stably maintained as long as no disruption occurs in the continual flow of solutions through the main channels (see Supporting Information).

We used two different methods (depending on the device design) to selectively localize the gels in the GGR (see Supporting Information). For devices with microchamber design (Figure 1A), the entire device was first filled with liquid gel followed by rapid aspiration with a house vacuum. Since the resistance across the GGR is considerably higher than that across the main channels, the gels in the GGR region were selectively left behind. The second method requires simple loading of liquid gel into the GGR inlet and can be used with the design shown in Figure 1B. To achieve reproducible results, the post structures (size and spacing) and surface properties of the device were optimized. After plasma treatment and bonding, channel surfaces were allowed to revert back to a hydrophobic state before the gel was injected into the GGR. The gel was confined to the GGR by surface tension until it solidified and then was used for gradient experiments. A number of gel materials such as collagen type I, Matrigel, and fibrin were successfully polymerized in the device using both methods.

This paper describes a microfluidic device capable of generating stable complex gradients across 2D surfaces and 3D gels in flow-free conditions. Two different designs and methods for generating selectively localized gels in micron-size channels have been developed. The ability to generate precise gradients in flow-free conditions and design different profiles by engineering the GGR chamber and placing them in series will be useful in a number of chemical (i.e., crystallization) and biological (i.e., tissue engineering and chemotaxis) experiments.

Supporting Information Available: Details of device fabrication, gradient characterization, a collagen gel micrograph, and gradient stability over time. This material is available free of charge via the Internet at http://pubs.acs.org.

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