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Microfluidic flow-free generation of chemical concentration gradients

Yao Zhou, Qiao Lin*

Department of Mechanical Engineering, Columbia University, New York, NY 10027, USA

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ABSTRACT

This paper presents a class of novel microfluidic concentration gradient generation (CGG) devices that create temporally stable chemical concentration gradients with complex shapes in a flow-free environment. The devices feature a two-layer channel design and the incorporation of a semipermeable membrane, which effectively segregates the concentration gradient region in the lower layer from the flow of reagent sample (simply "sample" onward) and buffer in the upper layer. In the mean time, free diffusion across the membrane constantly replenishes sample and buffer to maintain a stable concentration. The shapes of the concentration gradients are controlled by the geometries of the micro-channels and chambers. Concentration gradients with complex shapes can be achieved by piecewise combining constituent gradients with elementary shapes. Capable of generating concentration gradients in a flow-free environment, our devices eliminate undesirable flow stimulation on biological cells under investigation, while maintaining a stable chemical environment for cell studies.

1. Introduction

Chemical concentration gradients play an important role in cell growth and differentiation [1–3], signaling [4–6], chemotaxis [7–9], and other cell biology studies that involve exogenous chemical stimulation and cell response. Compared to conventional methods for generating chemical concentration gradients, microfluidic concentration gradient generation (CGG) device is of particular interest, thanks to their advantages in low reagent consumption and ease of control and automation. In stem cell research, for instance, microfluidic CGG was used to generate different gradient profiles of growth factors for controlling the growth and differentiation of human neural stem cells [10]. In pharmacological screening, gradients of drug molecules were also created using a microfluidic device for lead optimization in drug discovery processes [11]. In a high-throughput microfluidic cell culture array, the integration of a gradient generator enabled different cell lines to be cultured and treated with a variety of chemical concentrations in a single setup [12], substantiating the role of CGG devices in cell culture and treatment.

Currently, microfluidic CGG devices most commonly exploit molecular diffusion between multiple streams of laminar flow [7,8,10,13–17]. To generate a chemical gradient, streams of buffer and sample with different concentrations are introduced into a network of microchannels at carefully determined flow rates. The streams are joined at appropriately selected positions such that after the junctions, chemical molecules will diffuse between the streams for a predetermined time and distance depending on the flow rates. As a result, a desired concentration gradient profile will form across the channel width further downstream; the shape of the gradient profile is determined by a combination of the channel network configuration and sample concentration and flow rate of each stream. While such microfluidic devices using multi-stream laminar flow are capable of generating relatively complex gradient profiles, the presence of bulk fluid flow significantly limits their use in cell biology studies. For example, the bulk flow may introduce undesired shear force to cells under investigation, and the presence of continuous fluid flow may flush away cells that do not attach to surfaces. Even for adherent cells, cell-secreted growth factors essential for intercellular signaling may be carried away by fluid flow, leading to failure of signaling between cells. Moreover, the stability of the concentration gradient profiles is limited by flow rate stability; that is, as the shape of the gradient profiles critically depends on flow rates, it could be significantly changed by even slight disturbances to the flow. Finally, when relatively high flow rates are required, sample and reagent consumption becomes significant, especially when cells need to be cultured in the concentration gradients for extended period of time.

There have been a number of notable attempts to eliminate bulk fluid flow in microfluidic concentration gradient generation. For example, two parallel channels respectively containing sample and buffer solutions can be perpendicularly connected by another channel in which a linear gradient of the chemical concentration results [18]. Bulk flow is drastically reduced in the perpendicular gradient forming channel when identical flow rates were used

* Corresponding author. Tel.: +12128541906.
E-mail addresses: zhouyao@mit.edu (Y. Zhou), qlin@columbia.edu (Q. Lin).

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in the parallel sample and buffer channels, allowing the study of non-adherent cells. To generate concentration profiles more complex than linear gradient profiles, the shape of the gradient forming channel can be modified to generate nonlinear, yet monotonically varying gradient profiles [19]. However, such designs, relying on precisely matching sample and buffer flow rates, remain susceptible to mechanical disturbances to the microfluidic system.

Alternatively, a straight gradient forming channel lies between two large, stationary reservoirs, each respectively containing sample and buffer solutions [6]. The channel is coupled to the reservoirs through a semipermeable membrane, which eliminates bulk flow while allowing molecular diffusion. This approach is more effective in eliminating fluid disturbances in the gradient forming channel, but is limited to linear concentration profiles, which also diminish over an extended period of time. These limitations can be overcome by using a hydrogel as a medium in which concentration gradients are established [20,21]. For example, parallel sample and buffer streams may be separated by a sheet of hydrogel, through which sample molecules diffuse and a concentration gradient is created, and a gradient forming channel is placed in contact with the hydrogel to sample different concentration gradients depending on the location and shape of the channel [20]. This effectively eliminates flow disturbances in the gradient forming channel, and is capable of maintaining temporally non-diminishing gradient profiles with constant replenishment of sample and buffer, and by design of the gradient forming channel shape, allows generation of more complex concentration gradients. Unfortunately, due to slow molecular diffusion in hydrogels, this approach requires prolonged setup times for the concentration gradients (e.g., about 10 h [20]).

This paper presents an approach to microfluidic concentration gradient generation that is capable of generating temporally stable chemical concentration gradients with various shapes in a flow-free environment. The approach is based on two microfluidic layers separated by a semipermeable membrane. Bulk fluid flow of chemical sample and buffer solutions is contained within the upper layer, and chemical concentration gradients are generated within a flow-free microchamber in the lower layer. Cross-membrane diffusion of sample molecules allows continuous replenishment of sample and buffer from the upper-layer channels to the lower-layer gradient forming microchamber, whose shape can be designed to allow the generation of concentration gradient profiles of different shapes. Such flow-free gradient profiles will be useful for cell biology applications, especially those that involve non-attachable cells.

2. Principle and design

2.1. Generation of flow-free concentration gradients

Our approach to microfluidic concentration generation is based on a two-layer device configuration (Fig. 1). The upper layer consists of two parallel microchannels respectively containing a sample and a buffer solution, while the lower layer contains a gradient forming microchamber. The sample and buffer channels are connected to the microchamber through a semipermeable membrane, which is sandwiched between the two microfluidic layers, allowing molecular diffusion while preventing bulk fluid flow across the membrane. The microchamber is connected through the membrane pores to the sample and buffer channels at selected chamber boundaries (referred to as "control boundaries"). At these control boundaries, the fluid concentrations in the gradient forming microchamber are kept constant by the fluid flowing in the overlaying sample and buffer channels by means of molecular diffusion across the membrane at these boundaries.

During operation, sample and buffer are supplied in their respective channels at a flow rate enough for replenishment. At the control boundaries, due to the concentration difference across the membrane, sample molecules will diffuse from the sample channel into the gradient forming microchamber and then into the buffer channel. Fig. 1c illustrates the fluidic path in the sample and buffer channels, as well as the path for molecular diffusion across the membrane pores and the gradient forming microchamber. As sample and buffer are replenished at a constant flow rate in the upper layer, constant concentrations are imposed at the control boundaries of the gradient forming microchamber, with a higher concentration at the boundaries coupled to the sample channel and a lower concentration at those connected to the buffer channel. In the mean time, sample molecules also diffuse within the gradient forming microchamber. After an initial transition time, a concentration gradient will evolve and reach a steady state in the gradient forming microchamber. This gradient profile will not diminish over time thanks to the continuous replenishment of the sample and buffer solutions.

Due to the large cross-membrane flow resistance resulting from the small pore size, bulk flow is limited to the upper layer only, creating a flow-free environment in the gradient forming microchamber. The large cross-membrane flow resistance also makes the gradient forming microchamber virtually unaffected by flow disturbances inside the sample and buffer channels. As sample molecules inside the gradient forming microchamber move only through diffusion and not through convection, the resulting concentration gradient will not be distorted by fluid flow once the gradient is established.

2.2. Facilitating concentration gradient setup

In order to facilitate the establishment of concentration gradients, we did not solely rely on passive diffusion across the membrane during the initial gradient forming period. While setting up the gradient, we applied a higher pressure at the sample channel than the buffer channel, which, in the gradient forming chamber, created a very slight fluid movement carrying sample molecules in the same direction of molecular diffusion. This fluid movement was very slow due to the large fluidic resistance of the semipermeable membrane, so it would not cause potential disturbance to cells. When the front of the sample solution reached halfway through the gradient forming chamber, we changed both sample and buffer to the same flow rate to stop the fluid movement in the gradient forming chamber. Subsequently, molecular diffusion started to happen from the middle of the gradient forming chamber where the concentration gradient is the sharpest, and the gradient was developing in both directions along the chamber. This not only reduced the diffusion length L by half and hence diffusion time t by 75% ($t \sim L^2$), but also avoided the diffusion bottleneck at the semipermeable membranes.

Comparing to Ref. [20], we also used Alexa Fluor as the sample. However, we used water instead of hydrogel as medium, and the diffusivity of Alexa 488 in water is $D = 4.3e10^{-6}$ cm^2/s. Assuming we have the same length of concentration gradient generation chamber as in [20], i.e. 2.8 mm. Using our method, the diffusion length L becomes half of the chamber length, i.e. 1.4 mm. Thus, the diffusion time $t = L^2/2D = 2279$ s, i.e. 38 min (comparing to 10 h in [20]). Also, in our method, the concentration gradient across the membranes is almost zero while the gradient is developing from the middle of the gradient forming chamber, therefore the diffusion across the membrane is nearly negligible.

2.3. Control of concentration gradient shape

Concentration gradients with different shapes can be generated using different geometries of the sample and buffer channels and the gradient forming microchamber, combined with proper choice