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Selective and tunable gradient device for cell culture and chemotaxis study†

Dongshin Kim^{‡,a,b}, Mary A. Lokuta^c, Anna Huttenlocher^{c,d}, and David J. Beebe^{*,a,b}

^aDepartment of Mechanical Engineering, University of Wisconsin, Madison, WI, 53706, USA

^bDepartment of Biomedical Engineering, University of Wisconsin, 2142 Engineering Centers Building, 1550 Engineering Drive, Madison, WI, 53706, USA

^cDepartment of Pediatrics, 4205 Microbial Sciences Building, 1550 Linden Dr, Madison, WI, 53706, USA

^dDepartment of Pharmacology, University of Wisconsin, Madison, WI, 53706 USA

Abstract

This article describes a microfluidic device for cell culture and chemotaxis studies under various temporal and spatial concentration gradients of the medium or chemoattractant. Vertical membranes formed using *in situ* fabrication are employed to avoid fluid flow inside the cell observation chamber. Thus, the medium and chemoattractants are primarily provided by diffusion, maintaining cell–cell communication *via* secreted factors. Neutrophils were used to demonstrate the capability of the device for chemotaxis research. Experiments exhibited successful migration up a concentration gradient of interleukin 8.

Introduction

Chemotaxis is the directional movement of cells,¹ bacteria, and other organisms in response to certain chemicals in their environment. As an example, bacteria swim to either find food by directing themselves to the highest concentration of food molecules or get away from danger by moving away from high concentrations of repellants.^{2,3} Chemotaxis is thought to play an important role in disorders where cell motility is central to disease pathogenesis, including arthritis, cardiovascular disease and cancer. A typical example of chemotaxis is the motility of neutrophils directed by the cytokine interleukin 8 (IL-8).^{4,5} The motility of neutrophils is regulated by cytokines *in vitro* and has been of interest in chemotaxis research to understand the mechanism of interaction between chemoattractants and cells.^{6–8}

Recently, microfluidic devices for studying chemotaxis have been employed to collect more quantitative data and to take advantage of faster experiments, higher sensitivity, improved environmental control, and lower costs.^{6,9–11} Devices can be classified into two categories—those requiring a constant flow to shape and maintain the gradient and static generators that

†Electronic supplementary information (ESI) available: Device fabrication process (Fig. S1); preliminary cell motility test on polystyrene and OTS-treated glass surfaces (Fig. S2); displacement vectors of the twelve neutrophils in the field of view (Fig. S3); geometrical model of the fan-shaped chamber used for the FEM (Fig. S4); normalized concentration profile of the model during the first 10 min computed using MatLab PDETool (Fig. S5); video demonstrating neutrophil migration. See DOI: 10.1039/b901613a

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djbeebe@wisc.edu; Tel: (+608) 262-2260.

*Current address: Department of Physics, Brandeis University, 415 South Street, Waltham, MA 02453, USA.

create the gradient based solely on diffusion and use device geometry to shape the gradient profile.

In this article, we describe an *in situ* fabrication method to create a static microfluidic gradient generator for chemotaxis study. One of the major components of our device is a cell chamber which is divided by interfacially formed vertical membranes.¹² The vertical membranes provide improved access for imaging and allow for arbitrary membrane profiles. Their orientation also simplifies modeling since the diffusion boundaries are defined in two-dimensional fashion as compared to the previous design¹⁰ which uses horizontal membranes and has virtual diffusion boundaries. Thus, the device provides a controllable, predictable, and long lasting diffusion gradient profile which is generally not achieved by traditional methods.¹³⁻¹⁵ The well documented chemotaxis of neutrophils in response to IL-8 is used to demonstrate the system.

Methods

Design

A fan-shaped design was chosen to provide a monotonic concentration profile based on cylindrical coordinates (Fig. 1). It is composed of an observation chamber, two medium channels (source and sink), and two cell supply inlets. The observation chamber, located in the middle of the device, is separated from the medium channels by vertical membranes. The channels are used to introduce the organic and aqueous solutions that react to form the membranes during *in situ* interfacial fabrication.¹² After completion of the device, the medium channels were used to introduce media (with or without chemoattractant) into the observation chamber *via* diffusion. To maintain a constant concentration in the medium, it was necessary to exchange the medium periodically. In this experiment, the observation chamber was treated to have a hydrophobic surface because neutrophils have a natural tendency to adhere to hydrophilic surfaces hindering migration.¹⁶

Device fabrication was an adaptation of the process used in previous work.^{12,17-21} Detailed fabrication steps are described in the ESI.[†] The membrane pore size formed using the fabrication method employed in this paper are known to be smaller than 100 nm in diameter.¹²

One-dimensional gradient generation

The gradient generation of the fabricated device was validated using a fluorescent tracer (1 mM Fluorescein, Sigma Aldrich, St. Louis, MO). The device was filled with DI water and the fluorescent tracer was introduced into the source channel. Time lapse fluorescent images were taken using MetaMorph (v. 7.0 r2, Molecular Devices Corporation, Downingtown, PA) and analyzed using NIH ImageJ.

Cell migration experiment

For migration experiments, primary human neutrophils were obtained from healthy volunteers after informed consent and purified as described in a prior report.²² The device was filled with media (EGM-2MV, BioWhittaker Inc., Walkersville, MD) and as model cells, neutrophils were introduced in the observation chamber by passive pumping.²³ The device was incubated at 37 °C at 5% CO₂ for 30 min. Next, the mixture of IL-8 (12.5 nM) and fMLP (Formyl-methionyl-

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leucyl-phenylalanine, 125 nM) was introduced in the source channel by passive pumping. Time lapse images were viewed on an inverted fluorescent microscope (Eclipse TE300; Nikon, Melville, NY) and acquired using a cooled charge-coupled device camera (Hamamatsu Photonics, Hamamatsu City, Japan) at 20 $\times$. The time interval between images was 15 s and cells were tracked using MetaMorph and analyzed using NIH ImageJ.

Results

One-dimensional gradient generation

A one-dimensional concentration gradient in cylindrical coordinates was successfully generated using the fan-shaped device (Fig. 2 & 3). Fluorescein was introduced in the source channel by passive pumping to verify no flow into the observation chamber. The results indicated that a cylindrical concentration profile could be formed using the vertical membrane.

Calibration of the relative fluorescence intensity in the chamber can be achieved (as previously described^{10,24}) and is required for numerically accurate concentration values due to the non-uniform UV light illumination of the microscope and nonlinear response of the CCD camera to fluorescence intensities.

Cell migration experiment

The motility of neutrophils was comparable on both polystyrene and OTS-treated glass surfaces. ESI Fig. S2[†] shows the result of the preliminary cell motility test on both surfaces. Cell spreading was similar on both surfaces indicating that the motility on both surfaces was comparable as well (the motility of stimulated neutrophils is inversely related to the extent of cell spreading on materials¹⁶).

Neutrophils responded to the concentration gradient of chemoattractants generated by the device. The black arrow and white box in the right-hand image of Fig. 2 represent the orientation and position where the time lapse images were taken during the neutrophil migration experiment. Fig. 4 shows selected frames of neutrophil migration 30 min after the introduction of chemoattractants. The rounded neutrophils were polarized to have thin protrusions (lamellipodia) at the leading edge and a long tail (uropod) at the trailing edge as the concentration gradient of chemoattractants established. The longest distance one of the neutrophils travelled during 25 min was 162.5 μm toward the source. A movie file is available as ESI to show their migration.[†]

The x-axis shown in Fig. 4d was directed to the origin of the cylindrical coordinate, where the infinite source was located as indicated in the right-hand image of Fig. 2. All twelve neutrophils in the field of view responded to the concentration gradient and eleven out of twelve neutrophils (91.7%) migrated toward the higher concentration (see Fig. 5 and ESI Figure S3[†]). The sum of the twelve unit vectors computed based on the trajectories of the twelve neutrophils, pointed toward the source at an angle of -2.85° which indicated the majority of neutrophils migrated toward the source (see the gray vector in Fig. 5). A cell trajectory is often quantified using the persistence length which is the displacement vector of the cell trajectory divided by the total length of the cell trajectory.^{9,25} The average persistence length for the twelve neutrophils was computed to be 0.41 in the x direction and -0.03 in the y direction which confirmed the directionality of migrating neutrophils (see Table 1). The average velocity was 3.44 $\mu\text{m}/\text{min}$ in the x direction and 0.25 $\mu\text{m}/\text{min}$ in the y direction.

A theoretical model was developed as described in the ESI,[†] and qualitatively fitted with the cylindrical concentration profile of the gradient generator shown in Fig. 3. A fitted theoretical profile of the concentration gradient, computed using $0.2 \times 10^{-9} \text{ m}^2/\text{s}$ as the diffusion coefficient, is shown in ESI Fig. S5.[†] The fitted diffusion coefficient ($0.2 \times 10^{-9} \text{ m}^2/\text{s}$) was

41% of the known diffusion coefficient of Fluorescein in water.²⁶ The lower value is thought to be caused by the additional diffusion barrier due to the vertical membrane.

Discussion

The fan-shaped microdevice developed in this study can also provide a tool to examine spatial and cell–cell communication effects during migration in ways not previously possible. As cells migrate toward the source, they will aggregate due to the geometry of the chamber (Fig. 6a). In contrast, they will disperse when the source and sink channels are switched (Fig. 6b). The static nature of the gradient allows for the retention of cell–cell signaling *via* secreted factors. This provides additional functionality over flow-based gradient generators enabling an exploration of the effects of paracrine signaling on chemotaxis (*e.g.* the interaction between neutrophil recruitment to tissue wounding and the cross talk between immune cell activation and initiation of fibrotic responses in tissues).

Other potential applications include the study of quorum sensing. Quorum sensing is a gene regulation mechanism that bacteria use to accommodate the expression of certain genes in response to cell-to-cell signal molecules (CCSMs) such as indole and autoinducers that are believed to be cell-density-dependent.^{27,28} At certain level of cell population, bacteria start to express virulence genes that cause bacterial pathogens related diseases. Although it has been demonstrated that disruption of quorum sensing might be alternative methods to control infections caused by bacteria,^{29,30} CCSMs need to be clearly identified and more thoroughly studied to prove the relationship between the static the gene expression and bacterial cell density.³¹ The static gradient devices could provide methodologies both to study CCSMs and develop clinical strategies that may control quorum sensing and thus, prevent diseases.³²

Conclusions

Microfluidic cell assays which supply media or chemoattractants by diffusion were developed. The interruption of cell–cell communication, caused by the fluid flow and convection that usually occur in conventional methods, can be avoided or reduced. The capability of the device to generate concentration gradients was confirmed by both Fluorescein diffusion and neutrophil migration experiments.

The micro assay platform developed here will provide a useful tool for a wide range of basic and applied studies on cultured cells. For example, the developed method will provide convenient ways to investigate interesting cell migration behavior, including aggregation and dispersion under determinable concentration gradients while retaining paracrine signaling. By taking advantage of *in situ* interfacial fabrication, different shapes of multi-dimensional cell micro assays can also be realized in a simple fashion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Schematic diagram of micro assay using nylon membranes to create static gradient in cylindrical coordinates.

Fig. 2.

A fan-shaped concentration gradient device. Fluorescein was introduced in the source channel by passive pumping to verify no flow into the observation chamber. The black arrow and white box (right-hand image) represent the orientation and the position where the time lapse images were taken during neutrophils migration experiment.

(a) After 5 min

(b) After 10 min

Fig. 3.

Cylindrical concentration profile of the gradient generator. Images were captured during the first 10 min after the introduction of Fluorescein.

Fig. 4. Selected frames of neutrophil migration. Images were taken every 15 s; thus frame 100 is the scene after 25 min since frame 1. Migration traces are sketched with black lines. The scale bar in (d) indicates 25 μm . A movie file is available as ESI.[†]

Fig. 5.

The unit vectors of the 12 trajectories generated in Fig. 4. The gray vector indicates the sum of the 12 unit vectors. Two unit vectors at 353° and 351° are overlapped. The direction of 0° is aligned towards the source.

Fig. 6.
A schematic diagram of cell aggregation and dispersion.

Table 1

Average motility from the 12 trajectories shown in Fig. 4

Direction	Displacement (μm)	Persistence Length	Velocity ($\mu\text{m}/\text{min}$)
X	44.1	0.41	3.44
Y	-2.2	-0.03	0.25
S.D. ^a	39.9	0.21	3.98

^aStandard deviation of the vector.