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Review

Microfluidic tools for quantitative studies of eukaryotic chemotaxis

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ABSTRACT

Over the past decade, microfluidic techniques have been established as a versatile platform to perform live cell experiments under well-controlled conditions. To investigate the directional responses of cells, stable concentration profiles of chemotactic factors can be generated in microfluidic gradient mixers that provide a high degree of spatial control. However, the times for built-up and switching of gradient profiles are in general too slow to resolve the intracellular protein translocation events of directional sensing of eukaryotes. Here, we review an example of a conventional microfluidic gradient mixer as well as the novel flow photolysis technique that achieves an increased temporal resolution by combining the photo-activation of caged compounds with the advantages of microfluidic chambers.

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Introduction

Cell motility and chemotaxis are ubiquitous throughout the living world. They play an essential role for many biomedical processes ranging from embryonic development to immune response and tumor spreading (Horwitz and Parsons, 1999). Substantial progress has been made in the study of bacterial chemotaxis, where detailed knowledge of the underlying pathways and quantitative models are available (Berg, 2003). Signaling in eukaryotic cells, on the other hand, is more complex and, to date, much less well understood. Our knowledge of the chemotactic pathways that link the membrane receptor to the dynamics of the cytoskeletal machinery in eukaryotic cells is based on a number of selected model organisms like neutrophils or *Dictyostelium discoideum* (Van Haastert and Devreotes, 2004). In recent years our knowledge has advanced to the point, where quantitative tests need to be performed (Bagorda and Parent, 2008). Microfluidic flow can provide an exceptionally well controlled environment for such investigations.

To date, mathematical modeling focuses predominantly on phenomenological, low-dimensional descriptions as many molecular details of the chemotactic pathways still remain unknown. For example, a variety of models have been proposed to explain the initial stages of chemotactic signaling, generally referred to as directional sensing. They are based on different paradigms like the combination of a local activator with a rapidly diffusing inhibitory component (local excitation/global inhibition – LEGI – models) (Levchenko and Iglesias, 2002; Levine et al., 2006; Parent and Devreotes, 1999), on Turing-type instabilities (Meinhardt, 1999), or on bistable dynamics (Beta et al., 2008a; Mori et al., 2008). While all of these models show an initial symmetry breaking in a chemotactic cell, they predict different dose–response characteristics and dynamics for the internal asymmetry as a function of the external gradient signal. Thus, quantitative experimental tools are needed to test the modeling predictions in single cell studies with well-controlled external spatio-temporal gradient stimuli.

The classical setup to expose cells to a spatially non-uniform concentration field is the micropipette assay, a convenient qualitative test for directional responses. However, the chemoattractant gradient is poorly defined and changes in the course of time as more substance is diffusing out of the micropipette. Early efforts to generate spatially linear and temporally stable chemical gradients led to the development of diffusion based gradient chambers. A gradient is established by diffusion inside a porous medium or a small gap between two large reservoirs containing solutions of different concentrations. The most widely known versions of diffusion chambers are the Boyden (1962), Zigmond (1977), and Dunn chambers (Zicha et al., 1991). For a review of gradient techniques see Keenan and Folch (2008).

With the advent of microfluidic techniques, an entire new range of devices became available to perform highly controlled live cell stimulation experiments with high spatial and temporal resolution (Breslauer et al., 2006). In particular, with the invention of soft lithography (Xia and Whitesides, 1998), such devices became readily accessible to a wide community of users. Soft lithography is based on a two-step procedure: First, a master wafer is designed and generated based on standard photolithography. Second,

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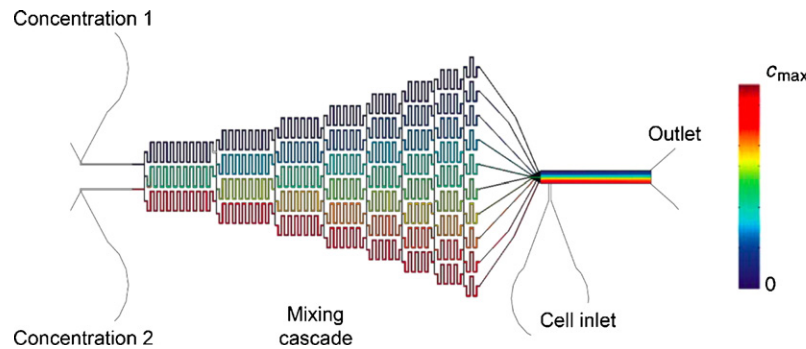

Fig. 1. Layout of the microfluidic gradient mixer used to generate a linear concentration gradient. The color-coding displays the concentration as obtained from a two-dimensional numerical simulation of the Navier–Stokes and convection–diffusion equation in the shown geometry using FEMlab 3.1. Black lines mark the in- and outlets that were not part of the numerical simulation. Parameters: density $\rho = 10^{-12}$ g/μm^3, kinematic viscosity $\nu = 10^6$ μm^2/s, inflow velocity $v = 3250$ μm/s, inflow concentrations: zero and $c_{max} = 2$ nM, cAMP diffusivity $D = 400$ μm^2/s, no-slip and isolating boundary conditions except for inlet and outlet.
Reproduced from Song et al. (2006).

molding of polydimethylsiloxane (PDMS) against the master wafer yields a microstructured polymer block that is sealed with a glass cover slip. Detailed, well-tested, and standardized protocols are available for this fabrication procedure (Whitesides et al., 2001). There are a number of outstanding advantages that make these devices an excellent tool for cell biological applications. They can be fabricated with a spatial resolution on the micron scale, so that the physicochemical environment of an individual cell can be controlled with great precision. Only a moderate level of equipment is necessary to fabricate such devices. The fabrication itself is straightforward, inexpensive, and reproducible. The devices are biocompatible and, due to the integrated cover slip, well-suited for optical microscopy.

Microfluidic chambers can be fabricated in a great variety of geometries and dimensions. They can be tailored according to the requirements of a specific experiment. On the one hand, many chemotaxis studies require long time migration experiments to gather sufficient statistics of the various motion parameters, but also to allow the study of temporal variability of the chemotactic parameters of individual cells. The latter is even more important as gene expression levels and consequently the chemotactic response may vary from cell to cell. Here, larger chambers are needed that exhibit sufficient long-term stability of a chemoattractant gradient. Such devices typically come with a relatively low time resolution (i.e. gradients change in minutes) but allow for the simultaneous observation of large cell populations (i.e. hundreds of cells). On the other hand, in order to investigate intracellular signaling in chemotactic cells, the experimental setup has to meet very different requirements. The first directional signaling events occur within only a few seconds after the cell is exposed to the external gradient stimulus. Such events are typically observed on the level of individual cells with the help of fluorescence techniques. Thus, single cell stimulation experiments with a high time resolution are necessary to investigate such processes.

In the following, we will exemplarily review two devices that meet these opposing demands, a classical microfluidic gradient mixer for long-term migration studies and the flow photolysis technique for rapid stimulation of individual cells.

The microfluidic gradient mixer

In 2000, the first microfluidic gradient mixer was introduced by Jeon et al. (2000). It is based on a pyramidal network of microfluidic channels that acts as a mixing cascade to generate a linear gradient between two arbitrarily chosen concentration levels. Solutions of the minimal and maximal concentration are introduced into the inlets and continuously pumped through the device. At each bifurcation in the pyramidal network, the flow is divided into an upper and a lower branch and diffusively mixed with the fluid from the neighboring channels. Consequently, a number of equidistant concentration levels are generated between the two input concentrations before all branches are finally merged in a single channel to produce a linear gradient that is oriented perpendicular to the direction of fluid flow, see Fig. 1 for an example of the layout. Since its invention ten years ago, this type of device has been used in various gradient studies (Jeon et al., 2002; Lin et al., 2004a; Wang et al., 2004). It has been modified and altered in various ways and became the paradigm of a microfluidic chemotaxis assay. One of the advantages of this flow device is that the gradient is stable over downstream distances of approx. 1 cm, which allows the investigation of large cell populations. In addition, the flow washes away all cell–cell signaling agents and thus provides an ideal environment for the study of solitary cell migration.

We have used a modified version of this gradient mixer to study the chemotactic behavior of *Dictyostelium discoideum* in stationary, linear gradients of cyclic adenosin 3′, 5′-monophosphate (cAMP) (Song et al., 2006). The layout of the microfluidic network that was used in this study can be seen in Fig. 1. We systematically investigated gradients ranging over eight orders of magnitude in steepness. Over more than three orders of magnitude, chemotactic responses were observed. In shallow gradients of less than 10^{-3} nM/μm, the cells showed no directional response and exhibited a constant basal motility. In steeper gradients, cells moved up the gradient on average. The chemotactic speed and the motility increased with increasing steepness up to a plateau at around 10^{-1} nM/μm. In even steeper gradients, above 10 nM/μm, the cells lost directionality and the motility returned to the sub-threshold level. The results are summarized in Fig. 2. Approximating the receptor binding by simple on/off kinetics, this data can be used to estimate the limiting receptor occupancies for *Dictyostelium* chemotaxis. Based on the K_d value of the *Dictyostelium* cAR1 receptor, it was found that in the regime of optimal response, the difference in receptor occupancy between the front and the back of the cell is on the order of 100 molecules only.

Microfluidic gradient generators operate under continuous flow conditions. They rely on the interplay of diffusive mixing and fluid flow to produce a well-defined, temporally stable concentration profile. In most cases, microfluidic devices are characterized for a fluid flow that runs through smooth channels and one may be tempted to assume this to be the environment of the cell. However, when applied in live cell investigations, these conditions are rarely fulfilled. Biological cells are three-dimensional, impermeable objects in the fluid flow. When attached to the sidewalls, they extend into the channel and perturb the flow field in their vicin-

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