Physica A 470 (2017) 132–145

Contents lists available at ScienceDirect

Physica A

journal homepage: www.elsevier.com/locate/physa

Generation and precise control of dynamic biochemical gradients for cellular assays

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HIGHLIGHTS

- A microfluidic device for diffusion-based gradient generation is presented.
- A unique design prevents the cross-flow between the source and sink channels.
- The device enables highly dynamic gradients in diffusion chambers.
- The device's capacity is demonstrated using yeast cells cultured in a gradient.

ARTICLE INFO

Article history:
Received 17 September 2016
Received in revised form 9 November 2016
Available online 5 December 2016

Keywords:
Microfluidics
Gradient
Diffusion
Yeast
Saccharomyces cerevisiae

ABSTRACT

Spatial gradients of diffusible signalling molecules play crucial roles in controlling diverse cellular behaviour such as cell differentiation, tissue patterning and chemotaxis. In this paper, we report the design and testing of a microfluidic device for diffusion-based gradient generation for cellular assays. A unique channel design of the device eliminates cross-flow between the source and sink channels, thereby stabilizing gradients by passive diffusion. The platform also enables quick and flexible control of chemical concentration that makes highly dynamic gradients in diffusion chambers. A model with the first approximation of diffusion and surface adsorption of molecules recapitulates the experimentally observed gradients. Budding yeast cells cultured in a gradient of a chemical inducer expressed a reporter fluorescence protein in a concentration-dependent manner. This microfluidic platform serves as a versatile prototype applicable to a broad range of biomedical investigations.

1. Introduction

Biochemical gradients are ubiquitous in biological systems. Gradients convey spatial and temporal information and function as cues for cellular decisions such as differentiation, proliferation and chemotaxis. Knowing how cells respond to them is thus fundamentally important to biomedicine and biotechnology applications. A well-studied example of biochemical gradients is that of morphogens that pattern embryonic tissues in a concentration-dependent manner (reviewed in [1–4]). Recent experimental evidence has suggested that the response of cells to morphogen gradients is regulated dynamically as the gradients change over time [2,4]. Dynamics of morphogen gradients are therefore crucial for tissue patterning, as corroborated by our theoretical study [5]. As biochemical gradients *in vivo* may never be static, investigations

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http://dx.doi.org/10.1016/j.physa.2016.11.134
0378-4371/

of cellular response to gradients require experimental means to control gradients in a dynamic manner to reproduce a natural cellular environment.

Microfluidic devices are becoming popular for studying gradients as they can generate and maintain desired gradient profiles stably for a long period [6–8]. A number of different designs of microfluidic gradient generators have been proposed, which can be grouped into two categories [8]: flow-based [9–13] and diffusion-based gradient generators [14–26]. The flow-based gradient is established by the diffusion at the interface of laminar flows. Diffusion-based designs utilize a passive diffusion between a source and a sink flow channel or reservoir of chemicals, which generates a linear gradient at steady states. Their advantages and disadvantages have previously been discussed [7,8]. For example, flow-based designs, unlike diffusion-based ones, can generate gradients of complex profile and maintain them stably for a prolonged time. The method of creating such non-linear gradients can reveal unexpected cell behaviour, for instance, in neutrophil chemotaxis [27,10].

One of the major drawbacks of the flow-based gradient generators for cellular assays is that cell-to-cell communication by diffusible factors is disrupted by continuous flow as such factors are washed away. Cells also experience a flow-induced shear stress, which may affect cell behaviour. Diffusion-based designs can overcome these problems and are appropriate for analysing the cell's response to gradients where autocrine or paracrine cell signalling plays an important role. A typical diffusion based design features rectangular chambers between a source and a sink flow channel. In devices with such a design [18,28], a technical difficulty is to minimize the unwanted cross-flow between the source and the sink to maintain a stable gradient [24]. Different schemes have been proposed to rectify this problem of flow-induced perturbation: membranes [14,20], hydrogels [17] or micropillars [19,22] between channels and a diffusion chamber; microfluidic "jets" that inject small amounts of fluid with minimal flow over cells [15]; increased flow resistance across the chamber by reducing its height [19]; embedding cells in a collagen matrix [28,19]; a contact zone to balance the pressures of the source and sink streams [16]; a two-layered device with micro-channels in the top layer and a buried diffusion chamber in the bottom layer [23].

Another notable solution for this technical challenge was put forward recently by Frank and Tay [24]. In their flow-switching device, only one side of the chamber is exposed to the flow channels at a time. By alternate switching of the on-chip membrane valves separating the chamber and the source/sink channels, gradients were established while a cross flow in the chamber was eliminated. Its capability of generating extremely stable spatial gradients was demonstrated with molecules such as lipopolysaccharides (LPS), tumour necrosis factor-α (TNF-α) and platelet-derived growth factors (PDGF) along with a fluorescent tracer (FITC-dextran, 40 kDa) [24].

The reliable maintenance of gradients by the flow-switching scheme, however, depends on the size of diffusing molecules (i.e., diffusion coefficients), the length of a chamber and the duration between valve switching, as gradients degrade by diffusion in a closed chamber. Our calculation suggests that in a chamber of 1 mm length with 60 s between flow-switching (as specified in [24]), molecular weights need to be larger than $\sim$3.5 kDa (Section 4); many biologically active molecules fall below this threshold. Microfluidic gradient devices for unicellular organisms like yeast or slime mould *Dictyostelium* usually have diffusion chambers of less than 1 mm in length (for example [18,21]). In a shorter chamber of 500 μm, unless using a shorter period of switching cycles, it may not be possible to maintain gradients of any biochemicals reliably with the flow-switching scheme (Section 4). The interval of flow-switching could be shortened in the flow-switching device for fast-diffusing (low molecular weight) molecules. Yet, it makes it necessary to reduce the number of parallel gradients that can be controlled in a single chip, compromising one of the very advantages of its design.

In this paper we used theoretical models and numerical simulations to aid the design and testing of a device for diffusion-based gradient generation. Our design has a channel junction upstream of the diffusion chambers, where the source and sink flows are merged to create a laminar flow away from the cell chambers; this enables precise balancing of flows in the source and sink channels thereby eliminating cross flows in the chambers. A similar idea was adopted in the device developed by Atencia et al. [23] and Irimia et al. [16]; their device also merges the source and sink flows separated by a laminar interface, which in turn generate a gradient in the diffusion chambers downstream. In their device, however, there is a diffusive mixing between the merged streams, which may be an issue depending on the length of the laminar interface and the flow rates. To avoid a diffusive mixing, our device has a thin separation wall that splits the merged source/sink flows towards the cell chambers. We also adopted the design features reported previously including chaotic mixer channels [29] with the so-called Dial-A-Wave (DAW) junction [30]. The modular combination of these preexisting microfluidics parts with the unique channel design allows rapid and independent control of biochemical concentration in the source/sink flows.

We demonstrate the capability of our device using fluorescent tracer dyes and yeast cells harbouring a fluorescent protein reporter gene that are exposed to a gradient of the inducer doxycycline (Dox). Unlike the flow-switching device, our design allows stable gradient generation irrespective of the molecular mass, as well as a rapid and dynamic control of gradients.

2. A model of diffusion-based gradient with adsorption

We consider the diffusion of molecules that can be adsorbed on the exposed surface (e.g. PDMS). The process of adsorption can be described by the law of Langmuir [31]. The theory of Langmuir assumes that molecules can only be adsorbed on the solid surface that are not yet covered by the molecules. Let ϕ be the fraction of surface already occupied by the molecules. The flux of molecules J_1 being adsorbed by the surface is:

$$J_1 = \mathrm{k}_1 u_f (1 - \phi), \tag{1}$$