Analytica Chimica Acta 743 (2012) 9–18

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

Review

Temporal gradients in microfluidic systems to probe cellular dynamics: A review

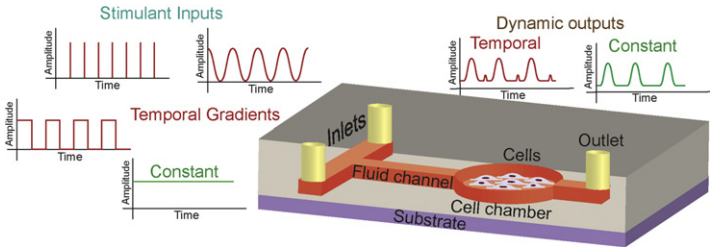
Raghuram Dhumpa[a], Michael G. Roper[a,b,*]

[a] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, United States
[b] Program in Molecular Biophysics, Florida State University, Tallahassee, FL, United States

HIGHLIGHTS

- Review article covering 2009–present.
- Topics include microfluidic devices capable of producing gradients with a focus on mammalian cells.
- Also included are selected examples of these waveforms on cell dynamics.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 4 July 2012
Accepted 4 July 2012
Available online 14 July 2012

Keywords:
Attenuation
Pulsing
Frequency response
Waveforms
Eukaryote

ABSTRACT

Microfluidic devices have found a unique place in cellular studies due to the ease of fabrication, their ability to provide long-term culture, or the seamless integration of downstream measurements into the devices. The accurate and precise control of fluid flows also allows unique stimulant profiles to be applied to cells that have been difficult to perform with conventional devices. In this review, we describe and provide examples of microfluidic systems that have been used to generate temporal gradients of stimulants, such as waveforms or pulses, and how these profiles have been used to produce biological insights into mammalian cells that are not typically revealed under static concentration gradients. We also discuss the inherent analytical challenges associated with producing and maintaining temporal gradients in these devices.

Contents

* Corresponding author at: Department of Chemistry and Biochemistry, Florida State University, 95 Chieftain Way, Dittmer Building, Tallahassee, FL 32306, United States. Tel.: +1 850 644 1846; fax: +1 850 644 8281.
E-mail address: roper@chem.fsu.edu (M.G. Roper).

0003-2670/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2012.07.006

Raghuram Dhumpa obtained his Bachelor degree in Electronics and Instrumentation engineering from Anna University (India) in 2005, his Master degree in Nanomaterials and Nanotechnology from Royal Institute of Technology (Sweden) in 2007, and received his Ph.D. from Technical University of Denmark (Denmark) in 2011. His research interests are in developing microfluidic systems and analytical methods to diagnose disease states and to study cellular dynamics.

Michael Roper received his B.S. in chemistry from the University of Texas at Austin, his Ph.D. from the University of Florida in 2003, and was a postdoctoral fellow at the University of Virginia until 2006. He has been an assistant professor at Florida State University since then and his current research interests include the development of microfluidic systems to investigate hormone release from islets of Langerhans.

1. Introduction

The *in vivo* environment that cells experience is complicated due to the number of signals that cells are exposed to, as well as the spatiotemporal profile of these signals. The temporal profile of many compounds *in vivo* is oscillatory, for example hormone secretion, for reasons that are only now beginning to be understood or investigated [1–3]. These dynamic signals may be used to increase the signal-to-noise ratio of the compound over the thousands of proteins found in serum [4] or reduce receptor desensitization [5]. *In vitro*, the time profile of stimulant delivery can affect the response of the system under investigation, highlighting the need for accurate reproduction by *in vitro* systems of the temporal patterns found *in vivo*. In some cases, delivery of temporally varying stimulus patterns allow observation and determination of intracellular dynamics or population behavior, both of which are difficult to observe under a non-changing stimulus.

Advances in understanding the temporal dynamics of cellular systems are dependent on the analytical devices used to deliver the stimulant waveforms. The ability to accurately and precisely control fluid flows affords microfluidic systems new avenues to study cellular dynamics that have not been possible using conventional macroscale devices. Numerous microfluidic devices have been applied in the field of diagnostics [6], cell biology [7], system biology [8], and synthetic biology [9]. Over the last decade, the popularity of using microfluidic devices shifted the paradigm from not only analyzing cellular targets, to now also controlling the cellular microenvironments. For example, a growing field in microfluidic research is the generation of spatial molecular gradients for studying cellular chemotaxis [10], morphogenesis [11], or electrotaxis [12]. Several comprehensive reviews have been written on spatial gradients and their applications in biology [11–14]. More pertinent to the topic of temporal gradients, was a 2008 review article by Jovic et al. that summarized how macro-systems have been used to generate temporal gradients and how the advent of microfluidics provided a more effective platform to probe cellular dynamics [15]. The authors emphasized the need for the development of next generation microfluidic platforms for studying cellular dynamics.

This review article is focused on advancements in the field of microfluidic systems since 2009 that use or generate temporal gradients to study cellular dynamics. The applications in this review were restricted to mammalian cells, with a recent review demonstrating systems for investigating the dynamics of yeast and bacterial cells [16]. Finally, this review article is not meant to be comprehensive, only a few representative examples have been highlighted to bring the importance of dynamic stimulations and the use of microfluidic systems to generate these profiles to the attention of the readers.

2. Temporal gradient generation

In recent years, there have been many microfluidic devices capable of generating temporal gradients that have been developed and used for cellular studies. As in most microfluidic systems, the devices have a characteristic length scale that is in the micrometer range where the fluid dynamics are dominated by viscous rather than inertial forces. At these scales, the flow is laminar where parallel streams of fluid mix only by diffusion at their boundary. A mixture of glass and plastic devices have been described with the majority of the ones mentioned in this review being either poly(dimethyl siloxane) (PDMS) or a combination of PDMS and glass.

2.1. Microfluidic devices used to generate temporal gradients

A typical microfluidic system used to generate temporal gradients delivers two or more analytes to a mixing channel where they mix to homogeneity prior to delivery to the cells under study [17]. To produce time varying patterns of reagents, the ratio of the two reagents are varied in time while maintaining a constant volumetric flow rate. The output concentration waveforms can be in the form of pulses, square waves, or sinusoidal waves depending on the application as will be discussed in the following sections. In Section 2.2, more information will be given for producing the correct waveform by optimizing the time the reagents spend in the mixing channel because too little time may not mix the reagents to homogeneity, while too much time may allow dispersion to have a detrimental effect on the shape of the waveform.

More elaborate methods to produce temporal waveforms have also been developed. For example, pulse code modulation (PCM) has been used to produce and deliver stimulant waveforms to ganglia of *Aplysia californica* [18] and also to murine islets of Langerhans [19–22]. In PCM, discrete pulses of analyte are introduced into a flowing stream of buffer where they broaden and mix due to dispersion, producing a homogeneous output concentration that is proportional to the temporal density of the analyte pulses [23,24]. For the device described in [20–22], two on-chip diaphragm pumps [25] were used to deliver pulses of glucose while also driving buffer through the microfluidic system. By varying the temporal density of the pulses, sine waves of fluorescein with various amplitudes