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Review

A review of chemical gradient systems for cell analysis

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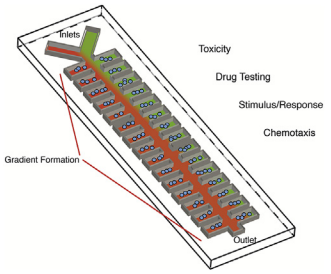
HIGHLIGHTS

- Microfluidic systems can achieve new gradient modalities.
- A review of gradient systems and applications.
- Microfluidic gradient generators can be applied to a wide range of bio-analytical problems.

GRAPHICAL ABSTRACT



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ABSTRACT

Microfluidic spatial and temporal gradient generators have played an important role in many biological assays such as in the analysis of wound healing, inflammation, and cancer metastasis. Chemical gradient systems can also be applied to other fields such as drug design, chemical synthesis, chemotaxis, etc. Microfluidic systems are particularly amenable to gradient formation, as the length scales used in chips enable fluid processes that cannot be conducted in bulk scale. In this review we discuss new microfluidic devices for gradient generation and applications of those systems in cell analysis.

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1. Introduction

In recent years microfluidic devices have been utilized in biomolecular and chemical gradient generation with increasing popularity. Concentration gradients within human body control various cell behaviors such as cell growth and differentiation [1–3], wound healing [4], inflammation [5], cancer metastasis [6], and drug delivery [7,8]. For example, biomolecular gradients have been implicated in tumor-cell invasion of metastatic cancer [9–12]. The migration and differentiation of cancer cells is driven largely by concentration gradients of attractant and repellant factors. Tumor cell chemotaxis has become a critical issue in the cancer drug discovery process and its study has been facilitated by gradient generators for some time [13–16].

Although microfluidic systems have had a large impact on cell biochemistry research in academia, the industrial sector has been slower to adopt microfluidic techniques. However, microfluidic chips could aid the drug discovery process, especially in concentration-based assays against the target, or in cells [16,17]. One of the most important tests to which microfluidic devices can be applied is in toxicity testing for a drug candidate, as most cytotoxic compounds have shown side effects against normal cells [18]. Microfluidic chips can also be used to test lead compounds for solubility, lipophilicity, diffusivity, stability, cytotoxicity, target specificity, tractability for synthesis, and effective and lethal concentrations [19–23].

One of the key benefits to using microfluidics is the ability to conduct single cell analysis [24]. Since cell signaling and molecular concentration levels change with time, it is difficult to understand individual cell response to different stimuli *in vivo*. However, recent advances in microfluidics have been able to address many of the limitations associated with conventional cell culture methods. As the size of microfluidic channels has decreased, chips have become well suited for fluid manipulation and analysis in cell based applications [25]. Most of the microfluidic gradient devices designed so far are made from PDMS (polydimethylsiloxane) polymer, which is biocompatible and air permeable [26]. Therefore, microfluidic devices can provide precise control over the culture environment that can be used for single cell analysis as well as for 3D cell culturing. Another distinct feature of microfluidics is the parallel processing. For instance, while creating a concentration gradient, cell response can be studied at the same time [27]. Microfluidic systems have been applied to many cell-based analysis, including ischemia/reperfusion [26], cell separation [28], chemotaxis [29], high throughput drug screening [30], and chemical or thermal gradient studies [23,31].

Most of the reviews on microfluidic gradient systems have highlighted biological applications of concentration gradient generation and limited emphasis on different types of gradient systems other than convection and diffusion based gradient systems.

This review provides a historical perspective of gradient generator development and focuses on their application to drug response in cell and other biological system. We also discuss the historical roots of gradient systems and present an overview of methods for the non-expert as well.

2. Historical perspective of concentration gradient generators

Before the emergence of microfluidic-gradient generators, conventional methods such as pipette injection, 96 well plates, diffusion through hydrogels, and diffusion chambers were used to create concentration gradients for cell analysis. Many of these developments are still in use, and often serve as inspiration for new microfluidic methods.

2.1. Chamber systems

Drugs that target cancer cell invasion have shown promise as anti-metastatic agents. Thus a better understanding of chemotaxis may provide information to find the correct target for the drug, a better drug, or appropriate patient selection in early clinical studies [32]. Cellular response to different concentrations of a lead compound may predict many drug related properties such as cytotoxicity and cell pathway inhibition. Transwell and Zigmond chambers have been widely used for over five decades for chemotaxis studies [33]. Subsequent improvements to the Transwell assay and Zigmond chambers resulted in the development of Dunn and Insall chambers [15,16]. These two chambers improved the image resolution and the long-term imaging capabilities of visual chemotaxis assays. Although all the chambers described here to assay chemotaxis can create gradients via molecular diffusion, their concentration gradient profiles cannot be maintained for long periods of time (<1–2 h) [15,16].

2.2. Pipette injection

The micropipette method is one of the most commonly used conventional methods used to create chemical gradients [34]. The main feature of this method is that in a chemotaxis assay one can move the pipette relative to the movement of cells. Micropipette injection methods are able to create stable gradients using pulsatile injection, although this approach is limited by the culture volume [34,35].

Pipette assays have shown significant benefits over chambers. Although Boyden, Zigmond, and Dunn chambers were able to create gradients with no liquid flow through the cell culture, gradients cannot be created/changed once the assay is started. However, micropipette assays are able to create gradients repeatedly by adding the test compound to the assay chamber. In addition, cells can be observed directly using fluorescence and confocal microscopy.

Chemical gradients can be readily generated and cell types such as neutrophils [36–38] and primary neurons [35,39] can be analyzed using micropipette methods. The gradient generated by the pneumatic injection depends on the hydrodynamic properties of the micropipette. These hydrodynamic properties are solely dependent on the shape of the pipette tip. Therefore, even when micropipette tips are pulled and operated under the same conditions, geometric differences between tips may cause different gradient profiles, leading to poor reproducibility.