Recent developments in microfluidic devices for *in vitro* cell culture for cell-biology research

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Microfluidics has developed as a powerful tool for cell-biology research due to its intrinsic advantages. Recently, considerable progress was made in microfluidic technology for cell culture and manipulation, and subsequent treatment and analysis.

We summarize microfluidic technologies that improve the efficiency of biological research at the microscale level. From a decade of research, we find that the full integration of microfluidics with cell biology and the development of a microscale cell-culture system show great promise for point-of-care diagnostics and high-throughput drug screening.

We discuss recent advances in technologies and methodologies in microfluidic cell culture and their cell-biology applications. We give some insights and directions for researchers interested in developing novel, robust microfluidic platforms for cell-biology research.

Keywords: Cell biology; Cell culture; Cell immobilization; Cell migration; Co-culture; Drug discovery; Gradient generator; Microfluidics; Micropatterning; Single cell

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1. Introduction

Cell-culture techniques that mimic complex *in vivo* microenvironments play important roles in cell biology, tissue engineering, and biomedical engineering. *In vivo*, cells are subjected to diverse physical or chemical signals, which vary in time and space, including gradients of cytokines, chemicals secretion from neighboring cells, physical interactions with extracellular matrix (ECM), and direct cell-cell contacts. So far, several conventional *in vitro* cell-culture techniques have been developed. However, these cells were grown in a static macroscale environment that greatly differs from the *in vivo* biological environment [1], so numerous aspects of cell function, including cell proliferation, differentiation, and migration, are affected to different degrees. Because of the strong dependence of the local environment on cell culture, it becomes extremely important to develop new technologies to control the cellular microenvironment precisely.

With the recent development of microfabrication methods and microfluidic technology, microfluidic systems are gradually being used as robust cell-culture tools for various cell-based assays. Microfluidics possesses a large number of advantages, including being easy to fabricate, low reagent consumption, parallel and rapid processing ability, and large-scale integration. However, the most favorable benefit of using microfluidics for biology research is the ability to control the local cellular microenvironment precisely without (or with less) interference from the external environment. A controlled cellular microenvironment enables better control of cellular behavior.

One effective strategy was through micropatterning the cells on extracellular matrices under microfluidic conditions. Also, the dimension of the microchannel at μm or sub-μm scale can accelerate changes of media, buffer, air and temperature, and also make single-cell handling and analysis more flexible. Microfluidic technology can study cell behaviors from single-cell to multi-cell levels, unlike conventional tools and methods.

Microfluidics has several biology-related applications (e.g., cell-migration studies, drug metabolism and toxicity testing, high-throughput drug screening through

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massive parallelization [2], single-cell or multi-cell manipulation and analysis, biosensing, and point-of-care diagnostics [3]). However, progress in using microplatforms in cell applications is at the proof-of-principle demonstration stage.

In this review, we mainly focus on the recent developments of technologies for *in vitro* cell culture and their cell-biology applications based on microfluidic techniques. Because the native environment is vital for cellular behavior, we first discuss how to design or to develop versatile microfluidic devices to mimic *in vivo* cellular microenvironments. Second, we discuss various microfluidic devices employed to generate different cell-culture formats [e.g., two-dimensional (2D) or three-dimensional (3D) cell culture, co-culture, single-cell culture and automatic cell culture]. Because microfluidics has several drawbacks for cell culture, we also discuss several effective strategies to solve those problems. Finally, we describe various biological applications, including high-throughput drug screening, cell-migration study and clinical diagnosis.

2. Microfabricated cell culture

In vitro cell culture has become one of the most important tools for modern biology research. Cells in all living tissues are interacts with the ECM and neighboring cells. Understanding the complexity of the cell microenvironment can provide guidance to mimic the conditions *in vivo* better. With tremendous advances, microfluidics has provided a versatile tool to mimic biological systems by systematically introducing various factors [4]. Also, it has the capability to create several conditions in the physiological range (e.g., mechanical strain and stresses through shear).

2.1. Controlling the cell microenviroment

The microenvironment of cells comprises complex chemical and mechanical parameters, including specific physicochemical properties (oxygen tension, temperature, pH, and osmolality), soluble factors, cell-cell contacts and cell-matrix interactions. These parameters provide a series of cues to regulate cell structure, function, and behavior. *In vivo*, the microenvironments surrounding cells are subject to dynamic changes (e.g., hormone levels and metabolism).

Unfortunately, conventional cell-culture techniques are not appropriate for transferring time-varying stimuli. *In vitro* dynamic soluble stimuli can be realized by careful, timely pipetting. However, pipetting is labor-intensive, and stimuli are removed by multiple wash steps during experiments.

Microfluidics possesses the ability to change the parameters dynamically and automatically, and individual environmental parameters can be fully controlled. Besides, most cells in the body are attached to the surrounding ECM for survival.

Furthermore, an important part of the environment is perturbation of chemical or mechanical signals to nearby cells, which often use signals themselves to regulate cell function and behavior. The formation of temporal and spatial concentration gradients of certain chemicals was very useful in many processes (e.g., chemotaxis studies [5,6], cell polarization, migration and communications). Various methods have been developed to generate quantifiable and controllable concentration gradients to mimic the *in vivo* environment.

Generally, the chemical gradients generated by microfluidics include static [7–10] and dynamic modes [11]. The static modes often rely on molecular diffusion in static liquid, so that continuous concentration gradients can be formed.

The formation of concentration gradients in dynamic modes is mainly based on the diffusion and the convection of flowing liquid in laminar flow. The gradient is often steady and well-quantified but with a limited narrow range.

Wang's group developed a radial micro-channel network to generate a combinatorial, quantitative and predictable concentration gradient with a wide range [12]. The radial channel network comprised multiple concentric circular channels and an increasing number of branch channels. The wide-range concentration gradient was formed by repeated splitting and mixing by perfusion culture of HeLa cells. However, direct fluid flow on cells may increase the shear stress to effect cellular physiological activity and wash out secreted factors. To overcome these limitations, it is necessary to generate stable static gradients to eliminate fluid shear stress. For example, Takayama's group developed a microfluidic device that could generate six distinct fluidic conditions containing three distinct steady-state linear gradients in six discrete cell chambers by integrating embedded normally-closed valves [13]. Because there was no flow in the cell-culture chambers, this device could be used for the analysis of sensitive or non-adherent cells.

Generally, the gradient is unstable and uncontrollable along with changes of space and time. Multiple chemical gradients with controlled spatiotemporal distribution in sub-cellular resolution could be generated by integration with a vertical membrane [14] or a biological barrier [15] in a microfluidic device. However, in several cell-biological events (e.g., cellular growth, differentiation, and proliferation process), cells often respond to non-linear concentration gradients of biochemicals. Khademhosseini's group designed a microfluidic device for generating non-linear concentration gradients containing exponential and sigmoidal types by exploiting diffusion of two aqueous species in an asymmetrical design to stimulate cells located within a microwell array [16].