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Cell Assays in Microfluidics

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Synonyms

Cell analysis/testing in microfluidic devices; Cell assays/analysis/testing on-chip; Phenotypic screening in microfluidic devices/on-chip

Definition

A *cell assay* is defined as measurement and analysis of cellular response to chemical and/or physical stimulus. Cellular responses are diverse: alterations of intracellular and extracellular biochemistry, cell morphology, motility, and growth properties. These responses characterize the cell *phenotype*, and are typically monitored in a culture dish or a multiwell plate, while more recently microfluidic devices have been employed. A cell assay performed in a microfluidic device is sometimes termed an *on-chip* assay.

► High throughput screening (HTS) is a class of analytical techniques in which many different assays are performed in parallel, or very rapidly in succession. Currently, there is great interest in performing cell assays in a HTS format.

Overview

Introduction to Cell Assays

The cell is the irreducible element of life and is often studied as a living model of complex biological systems. Some studies, such as analysis of cell morphology and growth, can only be evaluated by means of cell assays. Other studies, such as the analysis of cellular biochemistry, can also be performed by simpler, molecular assays, that is, measuring molecular interactions in cell-free systems. Molecular assays are faster and less complicated to perform but can result in misleading conclusions as it is impossible to mimic the complex and unique properties of the intracellular environment (organelle compartmentalization of reagents, spatially localized receptors, varying temporal expression, etc.). Thus, cell assays are preferable for study of living systems. Nevertheless, they have several disadvantages, including being expensive, time-consuming, and much more complex than other kinds of analyses. An additional drawback is that many cells, such as mammalian cell culture lines, require strict adherence to sterile operating conditions.

Cell assays are conventionally performed in culture dishes or in multiwell plates (plastic trays containing 96, 384, etc. wells). While culture dishes require milliliter volumes of media and reagents, multiwell plates contain microliter volumes and enable simultaneous analysis of multiple cell types or stimuli. Cell responses in microwell plates are often evaluated using microplate readers, which are specialized optical detection instruments designed to rapidly analyze all of the samples on a plate, making use of techniques such as fluorescence intensity and polarization, luminescence, or absorbance. Multiwell plates and plate readers have become the standard means for medium- and high-throughput screening in cell and biomolecular assays. In these experiments, the plates, the plate reader, the fluid handling apparatus, and other miscellaneous equipment are typically integrated in a robotic analysis platform. Two major drawbacks of robotic platforms are the expense of the instrumentation (over $500,000), and the cost of experimental consumables (e. g., plates, pipette tips, reagents, and cells), which can be prohibitive. (This is true in general, but even more so for cell assays). Hence, applications requiring high-throughput cell assays are out of reach of all but the wealthiest of laboratories.

Miniaturization of Cell Assays in Microfluidics

Microfluidics, a technology characterized by devices containing networks of micron-dimension channels, enables integration of multiple processes on a single platform while reducing reagent consumption and analysis time. As such, microfluidics has been touted as a solution for the challenges inherent in conducting cell assays in HTS format [1].

In addition to the potential for facilitating HTS, microfluidic devices are advantageous for cell assays for a variety of reasons, the most obvious of which is the similarity in dimensions of cells and microchannels (10 – 100 μm widths and depths). Another important advantage is flow: channels with this size are characterized by laminar flow where diffusion is the only source of non-axial mass transport. Although diffusion-based transport is slow for delivering reagents across long distances, in microchannels, diffusion enables rapid and repeatable reagent delivery. In addition, the combination of laminar flow and diffusion makes possible the formation of highly resolved chemical gradients across small distances; this feature is particularly useful for cell assays as such gradients are common in living systems but difficult to implement in macroscale setups. A fourth advantage of microfluidic devices is the increased surface-to-volume ratio which facilitates

favorable scaling of heat and mass transfer, as well as favorable scaling of electrical and magnetic fields that are often used in cell analysis. A final consequence of the size regime is in concentration of analytes: as cells in microchannels are confined in sub-microliter volumes, relevant analytes do not become too dilute and can thus be more readily detected.

Microfluidics does have some disadvantages for cell assays. One limitation is the difficulty inherent in controlling many reagents simultaneously. A potential solution to this limitation is the use of *digital* or droplet-based microfluidics (►digital microfluidics). A second limitation is that the high surface-to-volume ratio of microchannels enhances the adsorption of molecules onto channel walls, reducing the effective concentration of reagents, and potentially causing problems related to cross-contamination. Finally, a major challenge for microfluidic applications in general is the macro-to-micro interface. To take a full advantage of the reduced scale, new technologies are needed to enable delivery of very low volumes of cells and reagents into microfluidic devices.

Key Examples

Li and Harrison carried out the first cell assay in microchannels [2]. This seminal work made use of electrokinetically driven flow (electroosmosis and electrophoresis) to transport bacteria, yeast, and mammalian cells in channels and to implement low-volume chemical lysis (cell death). This theme of microfluidics-based cell transport, sorting, and lysis, has continued to be a popular application, as well as related work in using microfluidics to culture cells and to pattern them into structures. We acknowledge the utility of these methods (and note that they are featured in several good reviews – see El-Ali et al. [1] and other entries in the *Encyclopedia*), but focus here on describing microfluidics-based cell assays that fit the definition described above – application of a stimulus and measurement of a response. These assays fall into four broad themes sorted as a function of the type of response to be measured: intracellular biochemistry, extracellular biochemistry, mechanical properties, and electrical properties. Prior to discussing these kinds of assays, we describe the basic methodology common to all forms of cell assays in microfluidics devices.

Basic Methodology

Cell Culture

In a microfluidic device for cell-based assays, adequate culture conditions must be maintained for the duration of the experiment, which can span hours or days. While being cultured, cells must be continuously perfused with nutrients and oxygen; in addition, constant temperature and pH must be maintained. Cell populations exist either in suspension (dispersed in liquid media) or as adherent layers grown on surfaces presenting an appropriate matrix, (Fig. 1a). In practice, adherent cells must be detached from culture flasks and seeded into a microfluidic device while sufficient time has to be allowed to achieve proper cell attachment and reduction of stress induced by the transfer [3]. Mobile cells in suspension are easier to handle and require less time to adapt to the new environment.

Cell assays are either performed on cell populations or on single cells. Being naturally heterogeneous, cell populations are more physiologically relevant and observation of populations allows for analysis of intercellular signaling. However, some cell phenotypes are not observable in an averaged signal produced by the population, so these assays can only be implemented by studying single cells. This chapter deals mainly with analysis of cell populations in microfluidic devices; we refer the reader to other entries of the *Encyclopedia* that discuss single cell analysis (►single-cell analysis in microfluidics).

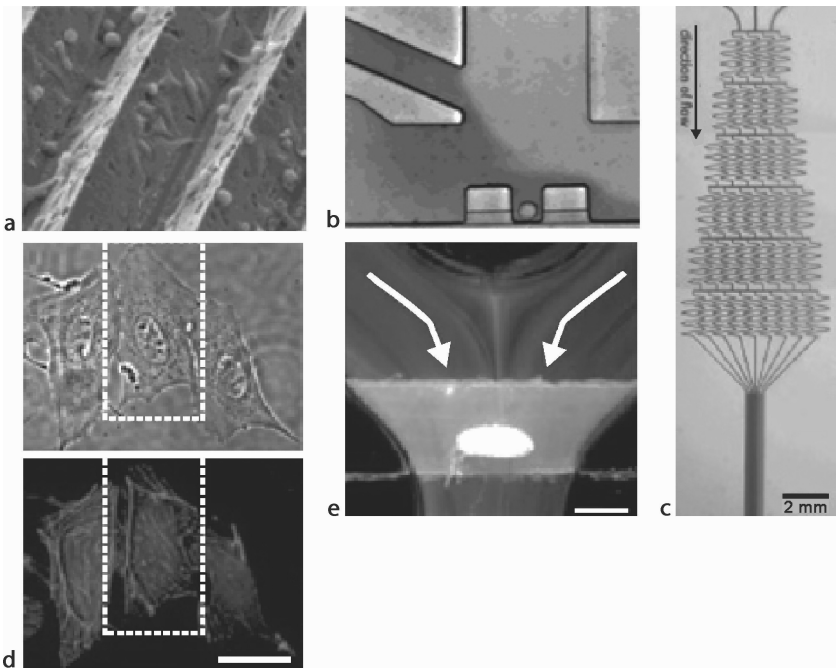
Microflow Design

With typical flow rates usually smaller than 10 μl/min, a microflow has to be capable of continuous and uniform perfusion of media as well as steady culturing conditions. The flow must have a distribution such that adherent cells are not exposed to significant shear stress. Cells in suspension are either assayed while carried by bulk microflow or, more often, after immobilization in the chip. Common immobilization techniques are hydrodynamic trapping [4, 5] and adsorbing cells to a chemically treated surface [6].

Microflow is realized either as electroosmotic flow (EOF) or pressure driven flow. EOF is in many cases not suitable for transport of cell media due to its high ionic strength; additionally, the electrical fields may cause adverse effects on a cell population. Thus, pressure driven flow generated by off-chip or on-chip pumps is preferable for cell assays. On-chip pumps are particularly convenient – of note, pumps formed by multilayer soft-lithography have been applied to analyzing T-cell behavior in microfluidic devices [4].

Materials

Microchannels are generally fabricated in silicon, glass or poly(dimethyl siloxane) (PDMS). Glass and PDMS provide transparency for optical monitoring, and the latter is also permeable to the oxygen and carbon dioxide that is



Cell Assays in Microfluidics, Figure 1 (**a**) HFF11 cell line grown in microfluidic V-channels for monitoring intracellular reporter gene activity. (Image reproduced, with permission, from [3]) (**b**) Cell from suspension hydrodynamically trapped in a perfusion chamber. (Image reproduced, with permission [4]) (**c**) A device for creating controlled linear, parabolic and periodic concentration gradients based on diffusive mixing (Image reproduced, with permission, from [7]) (**d**) Treatment of one part of an immobilized cell with latrunculin A and consequent disruption of actin filaments. (Image reproduced, with permission, from [10]) (**e**) *Drosophila* embryo developing while being exposed to two laminar streams of different temperature. (Image reproduced, with permission, from [11]) (▶ Color Plate 12)

necessary for cell culture. For long-term assays, thorough sterilization of channels is recommended to prevent cell contamination [3]. Assays involving adherent cells require microchannel walls to be pretreated usually by attaching extracellular matrix proteins (e. g., fibronectin, collagen, or laminin) to enhance adhesion. On the other hand, when transporting cells in suspension, channel walls are often coated with bovine serum albumin (BSA) to prevent non-specific adhesion [4].

Types of Stimuli

Stimuli used for cell assays in microfluidic devices can be categorized into two main groups:

- chemical stimuli, including drugs and other reagents, antibodies and gene transfection agents, and
- physical stimuli, including topographical features and other mechanical and electrical forces.

Both kinds of stimuli are translated by cell signaling networks into a diverse range of responses, including growth and proliferation, migration, activation of metabolic pathways, production and release of proteins and other biomolecules, and the initiation of apoptosis (programmed cell death).

Detection Methods

Cell responses to physical or chemical cues are measured in microfluidic devices primarily via optical or electrochemical means. Fluorescence is the most widely used optical detection technique, because absorbance detection (commonly used for macro-scale assays) is of limited value in microchannels because of the short path lengths. Fluorescence detection, characterized by its unparalleled sensitivity, is easy to implement in microfluidic systems. Chemiluminescence and bioluminescence also offer low detection limits and have less background noise than fluorescence [6]. Electrochemical detectors are even more easily integrated with microfluidic devices and often are much less expensive than optical systems. However, fabrication of electrodes is a technical challenge and the electrical fields used in detection can interfere with on-chip processes such as electrophoresis. Electrochemical techniques include potentiometry, amperometry and con-

ductometry. Recently, mass spectrometry detectors and nuclear magnetic resonance detectors have been interfaced with microfluidics as well.

Key Research Findings

Cell assays in microfluidics can be classified into four themes: intracellular biochemistry, extracellular biochemistry, mechanical properties, and electrical properties.

Intracellular Biochemistry

Intracellular biochemistry assays were the first, and continue to be the most common cell assays performed in microfluidic devices. Controllable and highly resolved delivery of reagents to cells and facile integration with optical and electrochemical detection techniques has sparked research in many areas, ranging from metabolism to gene expression analysis. We categorize these assays in terms of the type of chemical stimuli (homogeneous vs. heterogenous), as well as by assay duration and throughput.

Homogeneous Stimuli

In the most straightforward form of intracellular biochemistry assays, a homogeneous stimulus is applied to cells, after which various indicators of metabolism or signaling are measured. Wheeler et al [4] performed cell viability assays and measured intracellular Ca^{2+} flux in Jurkat T-cells and U937 cells. A suspension of cells was hydrodynamically manipulated and focused onto a dock at a T-junction stagnation point (Fig. 1b). The integrated device, which relied on multiple on-chip valves and pumps, enabled a high degree of control of reagent delivery while facilitating complete solution change in approximately 100 ms.

Heterogeneous Stimuli

While homogeneous stimulus-response assays are more straightforward, a particularly useful kind of assay relies on heterogenous stimuli in the form of chemical gradients. A device for creating controlled linear, parabolic and periodic concentration gradients was developed by Dertinger et al. [7], employing diffusive mixing in a complex network of serpentine microchannels (Fig. 1c). This kind of structure has been exploited widely in microfluidic cell-based assays. For example, Thompson et al. [8] stimulated HeLa S3 cells with 8 different concentrations of the inflammatory cytokine TNF-α (an intercellular chemical messenger protein), and monitored the resulting dynamic expression of the NF-κB transcription factor with a fluorescent reporter. Another promising application of controllable concentration gradients was reported in studies of stem cells [9] – this is particularly relevant, as stem cell differentiation is highly dependent on microenvironmental cues.

In addition to forming gradients, multiple laminar streams can provide stimuli selectively to subcellular domains. In a striking example of this phenomenon, Takayama et al. [10] delivered a fluorescent dye specific for mitochondria to a designated portion of a cell. After staining, the migration of labeled mitochondria was observed within few hours. In the same study, local cytoskeletal structures were similarly effected by delivering latrunculin A (an agent that promotes dissolution of actin filaments), which resulted in localized disruption of cell morphology (Fig. 1d). Similarly, different temperature stimuli were applied using laminar streams to deliver fluid to immobilized *Drosophila* embryos (Fig. 1e) [11]. Posterior and anterior parts of embryos treated in this manner exhibited different development as a function of the different temperatures applied.

Long-Term Assays

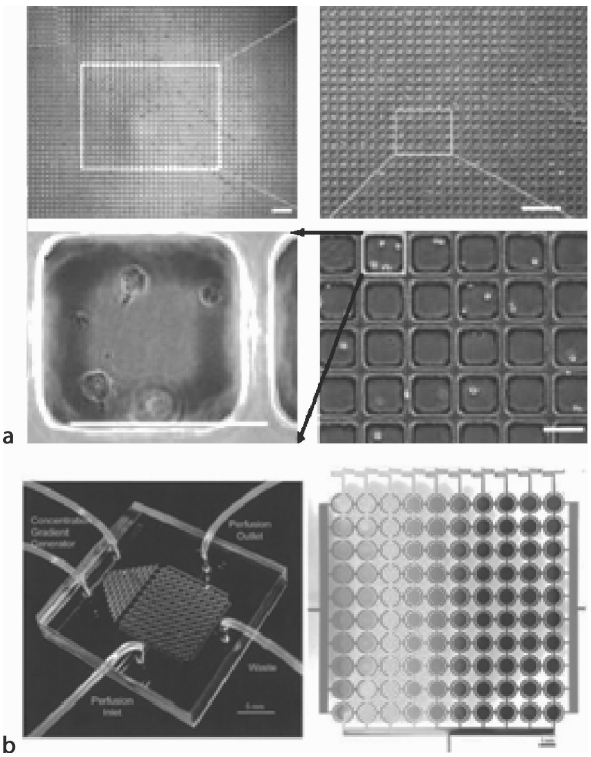
Most of the aforementioned work describes short-term assays that were largely independent of cell response to the microfluidic environment. A thorough investigation of a microfluidic system for long-term experiments was performed by Davidsson et al. [3] using luciferase (an enzyme that catalyzes bioluminescent reactions) reporter gene activity in the cell line HFF11. Cells were immobilized on silicon chips at 37 °C (Fig. 1a), incubated in CO_2-independent cell media, and monitored for up to 30 hours. The authors observed that cell stress and consequent non-specific gene expression were caused by

- detachment and transfer of adherent cells from culture flask to the chip,
- a change of environment from static to flow, and
- changes in chemical environment (e. g., addition of fresh cell media.)

By allowing cells to incubate and adapt to new conditions for several hours, non-specific gene expression decreased to a low, steady level.

HTS Assays

Although a few studies have described medium-throughput microfluidic cell assays, the potential of this application has not yet been fully realized. Quake and coworkers [12] developed a device comprising hundreds of reaction chambers, a complex network of microchannels, and multiplexed valves. The utility of the device was illustrated by detecting a recombinant cytochrome c peroxidase (an enzyme used for chemiluminescent detection) expressed in *E. coli*. This device, which houses 256 725 nL chambers, demonstrates the potential for high-throughput



Cell Assays in Microfluidics, Figure 2 (**a**) Microfabricated array of cell chambers for high-throughput screening. (Image reproduced, with permission, from [13]) (**b**) Microfluidic array for high-throughput cell assays with concentration gradients formed across ten columns. (Image reproduced, with permission, from [14]) (▶ Color Plate 17)

cell assays with minimal reagent use. A similar platform was developed by Chin et al. [13], and contains 10,000 microwells (Fig. 2a). Rat neural stem cells were seeded in the wells and enclosed in a microfluidic system such that all cells shared the same media, including any chemicals secreted by the cells. Proliferation of over 3,000 individual cells was monitored over period of days in single-, two- and three-cell-per-well assays. Hung et al. [14] developed a device for conducting cell assays in 100 chambers (Fig. 2b). In addition to perfusing cells with fresh media and assay reagents, the device was capable of passaging cells, i. e., growing multiple generations after cell division. Although these studies are promising, microfluidic systems capable of achieving throughput similar to conventional robotic platforms have not yet been realized.

Extracellular Biochemistry

Cells secrete signaling molecules (e. g., hormones and neurotransmitters), growth factors, and metabolic products. Measurements of the secreted molecules are required for studying cell–cell communication and regulation of cell secretory processes. Conventionally, these measurements are implemented by perfusing cells with chemical stimuli, collecting the perfusate, and analyzing it by *immunoassays* or other means. Integration of these processes on-chip enables continuous, automated monitoring and analysis of cellular secretion with high temporal resolution. This capacity is required to observe a complete secretion profile of system with complex kinetics. For example, insulin secretion goes through several phases, including initial burst, plateau and oscillations, all within seconds.

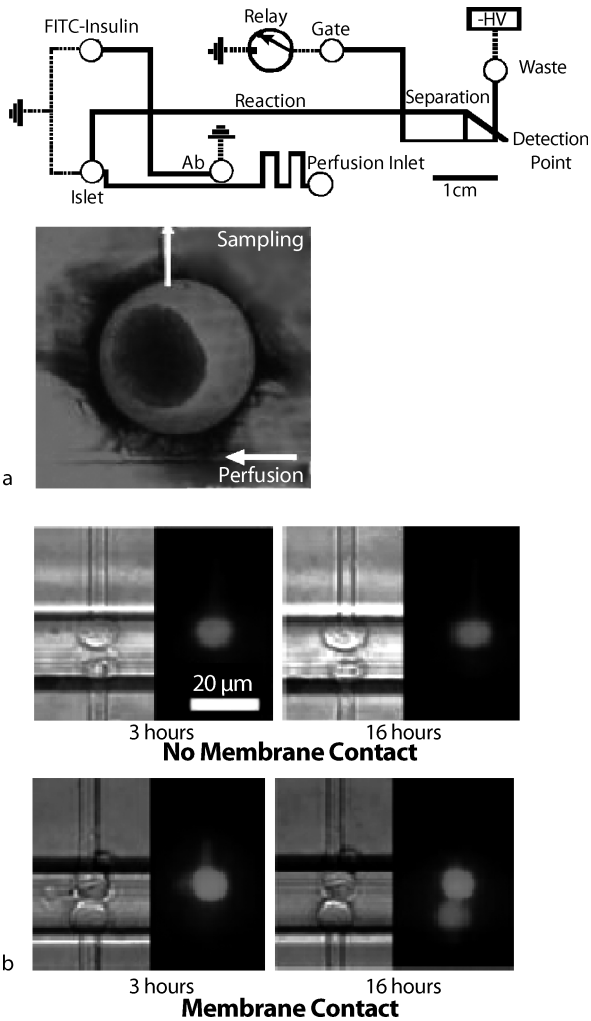
Understanding the mechanism of insulin secretion is tremendously important in diabetes research. In response to this challenge, an on-chip system for monitoring insulin secretion from islets of Langerhans (groups of pancreatic beta cells) was developed by Kennedy and coworkers [15]. The device enables a fully integrated cell assay with continuous on-chip cell perfusion, sampling of secretions, downstream mixing of labeling reagents, and separation and analysis with high temporal resolution (Fig. 3a). Islets were stimulated with glucose and extracellular fluid was sampled and analyzed every 6 s by means of an electrophoresis-based immunoassay having a 0.8 nM detection limit. Cells exhibited no morphological changes during the two-hour assay.

While the methods and devices developed by the Kennedy group for analysis of insulin secretion are the most comprehensive of this type, several on-chip methods have been reported for other analytes. For example, Davidsson et al. [6] designed a system for quantitative analysis of secreted glucose and ethanol. The allergic response of rat leukemia cells was monitored in a microfluidic device [16] by measuring fluorescence from a dye released along with histamine after stimulation. Lee et al. [5] investigated cell–cell communication via gap junction interactions on single cell pairs. The authors trapped two different mouse fibroblast populations at opposite sides of a microchannel and detected the interaction between cells by monitoring dye transfer between them. If the trapped cells were in contact, the dye was transferred within 16 h (Fig. 3b).

As these studies demonstrate, microfluidic devices are a useful and in some cases unique platform for dynamic, high-resolution measurements of cell secretory profiles. Relative to conventional tools, these on-chip methods enable superior control of perfusion, high-frequency analyte sampling, and integration of various processes on a single device.

Mechanical Response

Mechanical responses of cells to physical or chemical stimuli include cell growth patterns, morphology, adhesion and motility. Many mammalian cells grown in culture are adherent. For such cells, the adhesive interactions with the



Cell Assays in Microfluidics, Figure 3 (**a**) Schematic of a device for continuous perfusion of pancreatic cell islets and stimulation with glucose, and monitoring of insulin release (top). Perfusion chamber with an islet (bottom). (Image reproduced, with permission, from [15]) (**b**) Microfluidic device for monitoring cell–cell communication via gap junctions. Fluorescent dye transfers from one cell to another only when cells are in contact. (Image reproduced, with permission, from [5])

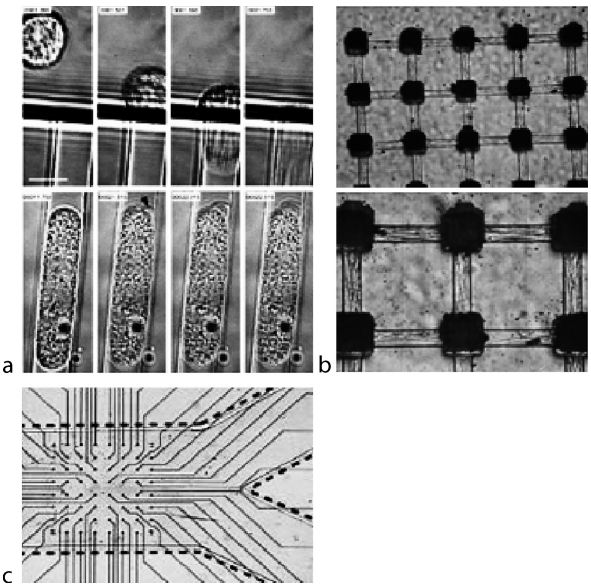
physical environment (extracellular matrix or other cells) are a critical determinant of their biochemistry and behavior. Microfluidics has become a useful tool for studying these interactions. Many of these studies are reviewed elsewhere (►cell adhesion and detachment), so we focus here on assays that evaluate the effects of physical or chemical cues on cell morphology or motility.

Cell Morphology

Cell morphology, or shape, is important for survival, differentiation and migration. A few microfluidic cell assays have been conducted to characterize cell elongation, contraction and other morphological changes in response to mechanical forces and chemical cues. Gray et al. [17] studied endothelial cell shape in microchannels under static and dynamic flow conditions. Under both conditions significant cell elongation in the direction of a microchannel/flow was observed after 12 h. In other study, Li et al. [18] used a microfluidic platform and an acoustic wave sensor to monitor contractions of heart muscle cells. In addition to the effects of chemical stimuli, alterations in contractions were observed to be a function of electrode size, presence of the microchannel plate, and liquid loading onto the sensor.

Cell Motility

In cell motility and migration studies, special attention has been paid to chemotaxis, or migration of cells in response to gradients of chemicals known as chemokines. Chemotaxis of human neutrophils (immune cells that migrate to sites of infection or injury) was evaluated in seminal work by Jeon et al. [19]. Complex gradients of the chemokine interleukin-8 were generated in microchannels and maintained at a steady state using the pyramidal branched microchannel structure [7] described above. Others have noted that when generating chemokine gradients, care should be taken to moderate flow rates as a bias in cell migration is observed under high shear flow [20]. Beebe and coworkers [21] monitored neutrophil chemotaxis under concentration gradients formed statically, i. e., without flowing fluid. Chemical species diffused into a cell microchamber through a membrane which imposed high fluid resistance to minimize convective flows. In this manner, soluble cues including autocrine factors (secreted by a cell itself) and paracrine factors (secreted by other cells) were not washed away with a laminar flow but rather accumulated in the microenvironment. This design allows for examining the influence of cell–cell communication on chemotaxis or on other cell responses to chemical stimuli. While movement of cells in response to chemical factors has been a popular research topic, only a few studies have evaluated the effects on cell motility of mechanical stimuli. In one important study [22], neutrophil morphology, rheology and viscoelasticity were monitored when cells were seeded in microchannels of smaller dimensions than cell diameter (Fig. 4a). An important observation was made in that mechanical deformation of neutrophils is not passive, but rather active, as migration capabilities are enhanced through the formation of pseudopods and a reduction in cell stiffness. Undoubtedly, these findings encourage further research in this field.



Cell Assays in Microfluidics, Figure 4 (**a**) A neutrophil flowing into a microchannel and becoming deformed. After entering the channel, the cell forms a pseudopod (arrow) that facilitates movement. (Image reproduced, with permission, from [22]) (**b**) Neurons seeded in chambers on top of patterned electrodes, with cell structures protruding into microchannels. (Image reproduced, with permission, from [24]) (**c**) Microfluidic channel (dashed lines) fabricated on top of patterned electrodes for recording neuronal activity. (Image reproduced, with permission, from [25])

True cell mechanical stimuli can hardly be implemented in macro-scale assays. Therefore, most of the work prior to the advent of microfluidics was focused on cellular response to chemicals or soluble signals. In contrast, as illustrated by the studies above, microfluidic devices are capable of mimicking in vivo physical conditions including topography and shear stress, which enables new research focused on mechanical response of cells to mechanical stimuli in combination with chemical cues.

Electrical Response

Electrical measurements on cells are discussed in other entries in the *Encyclopedia* (►cell electrical measurements, ►patch-clamp measurements on-chip). We focus here on the basic cell electrical responses assayed in microchannels, most of which have been developed in the context of cell-based biosensors. These studies typically evaluate cell action potential which is a net electrical change in cells resulting from changing concentrations of intracellular ions. Action potentials are important physiologically because they result in the transmission of signals between nerve cells and contraction of muscle cells. These electrogenic cells are very sensitive to chemical changes in the cell microenvironment and changes in action potential are detectable much sooner than any morphological changes.

DeBusschere and Kovacs [23] developed a portable microfluidic platform integrated with a complementary metal-oxide semiconductor (CMOS) chip which enables control of temperature as well as the capacity to measure action potentials in cardiomyocytes. When cells were stimulated with nifedipine (a calcium channel blocker), action potential activity was interrupted. Morin et al. [24] seeded neurons in an array of chambers in a microfluidic network integrated with an array of electrodes (Fig. 4b). The electrical activity of cells triggered with an electrical stimulus was monitored for several weeks. Cells in all chambers responded asynchronously to the stimulus. This device illustrates the utility of microfluidic tools that can investigate structure, function and organization of biological neural networks. A similar study probed the electrical characteristics of neurons as they responded to thermal stimulation [25] in a microfluidic laminar flow. Neurons were seeded on an array of electrodes (Fig. 4c) which allowed for measurements of variations in action potentials when cells were exposed to different temperatures.

By combining facile electrical measurements with controllable physical and chemical stimuli at the micron level, microfluidic devices are developing into a versatile platform for cell-based biosensors, drug discovery, genetic analysis and diagnostics.

Future Directions for Research

The studies summarized here were selected to demonstrate the potential of microfluidics for use in cell-based assays. We do note that the majority of the methods described are in a proof-of-principle stage. For real-world application in both academic and industrial research, a few hurdles must be overcome including device reliability, integration and automation, throughput, and macro-to-micro interfacing. We believe these challenges are solvable, and given the trajectory of interest and innovation in this area, we speculate that microfluidic cell assay tools will soon become irreplaceable in biosensing, diagnostics, drug screening and other biomedical applications.

Cross References

- ► Cell Adhesion and Detachment
- ► Cell Culture (2D and 3D) On Chip
- ► Electrokinetic Motion of Cells and Nonpolarizable Particles
- ► Cell Patterning on Chip
- ► Cell Sorting
- ► Single-Cell Analysis in Microfluidics

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Cell-Attached (Single-Channel) Voltage Clamp

► Patch Clamp Measurements On-Chip

Cell Attachment

► Cell Adhesion and Detachment

Cell-Based High-Throughput Assays

► Microfluidic Systems for High-Throughput Screening

Cell Binding

► Cell Adhesion and Detachment

Cell Capture

► Cell Adhesion and Detachment