Chapter 11

Microfluidic Probes to Process Surfaces, Cells, and Tissues

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Introduction

Over the last two decades, microfluidics has made significant contributions in cell biology research and tissue engineering by providing novel approaches and methods. Microfluidics is defined as the manipulation of fluidics at the micro-scale,[1] where the physics of fluid behavior is dominated by different phenomena than at the macro-scale, yet predictable and controllable.[2]

In this chapter, we briefly discuss the use of microfluidic devices in biological research, and highlight their use in processing surfaces, cells, and tissues. We explain the advantages that microfluidic devices can bring to biological experiments in comparison to the conventional methods such as Petri dishes, flasks, and microtiter plates (Fig. 1(a)). We also describe the limitations and challenges that are associated with microfluidic devices, mainly because of their closed-channel configuration. A microfluidic device can be described as a network of closed channels and chambers where at least one of the dimensions is

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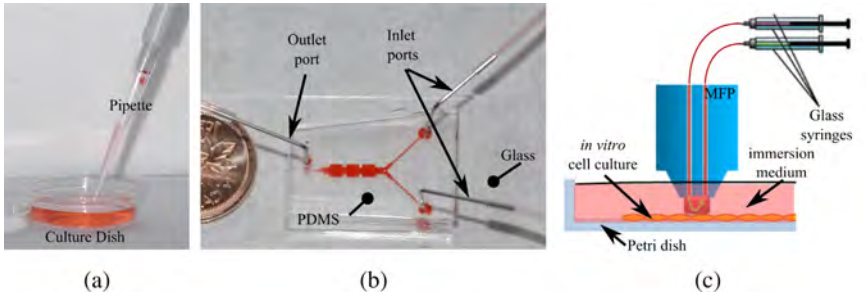

Fig. 1. Traditional and microfluidic methods for *in vitro* cell culture. (a) A conventional technique for culturing and stimulating a population of adherent cells. (b) A microfluidic device that encloses a network of closed channels and chambers with a set of input/output fluidic connections. The device is made out of a Polydimethylsiloxane (PDMS) layer that is bonded to a glass substrate. The channels are filled with red food dye to facilitate visualization. (c) Schematics of the channel-free microfluidic probe (MFP) operating on top of a substrate (i.e. Petri dish) and immersed in liquid. (Reproduced with permission of the Royal Society of Chemistry.[3])

less than 1 mm, and a set of fluidic access points to the network, which represent the fluid flow inlets and outlets (Fig. 1(b)).

The focus of this chapter is the microfluidic probe (MFP) and its biological applications. The MFP works in an open configuration and can be moved to areas of interest, thus can overcome various limitations that are associated with the use of microfluidic devices with closed channels (Fig. 1(c)). The MFPs are envisioned to be the next generation of microfluidic devices, and can be coupled with the traditional culture protocols and other technologies. Through the following sections, we emphasize on the use of MFPs in processing surfaces, cells, and tissues.

1. Microfluidic Devices for Biological Research

Microfluidic systems have many advantages that overcome the conventional tools used in biology laboratories. Notably, microfluidic systems offer miniaturization of the experimental footprint, speeding up of experiments, parallelization and thus high-throughput readout, reduced sample consumption, improved efficiency and robustness of experimentation by providing predictable, repeatable, and controllable

fluidic behaviors and conditions.[4,5] As a result, microfluidic systems have become increasingly prevalent and have revolutionized numerous areas in biomedical research. To name a few, microfluidic devices have been used for the study of cell signaling[6] and fusion,[7] the study of cellular proteomics[8,9] and genomics,[10] the study of cellular mechanics[11] and dynamics,[12,13] and the study of cellular chemotaxis and electrotaxis.[14] Other areas where microfluidic devices are being developed and applied are the fields of tissue engineering,[15,16] surface patterning,[17–19] point of care diagnostics,[20,21] and drug discovery.[22,23] The efforts to develop robust microfluidic systems for these research areas and many others are currently performed by a vast number of groups around the world and commercialization of microfluidics has gained magnitude in the global market.[24–26]

Despite the powerful features that microfluidics bring, they entail different challenges and limitations, which can be attributed mainly to the materials employed to fabricate these chips and the closed channel configuration. Microfluidic chips are generally composed of a network of channels and chambers where their dimensions range from tens of micrometers to a few millimeters.[1,2] Based on the desired application and functionality, the dimensions and complexity of the microfluidic chips vary. Microfluidic chips are typically composed of a layer of polydimethylsiloxane (PDMS), which is a silicon-based organic polymer,[27] and a glass substrate (Fig. 1(b)). Patterning of PDMS can be achieved using the different photo- and soft-lithography techniques[28,29] or by using other micromachining methods.[30]

The miniaturized size of microfluidic networks is associated with high flow resistance that prevents rapid reagent exchange, consequentially the introduction of biological samples (such as cells) within the network is challenging. Furthermore, some regions within the network cannot easily or selectively be accessed as in open-surface setups.[31,32] Indeed, these issues become even more critical when aiming to enclose large biological samples inside the chip, which limits the use of these devices with samples such as embryos or tissue slices. Beyond the issues of closed microfluidic systems, the switch from performing experiments using the traditional cell culture protocols (i.e. Petri dishes) to applying them to the micro-scale systems is not straightforward. Cells

in the microscale devices experience different physical environments than those present at the macro-scale.[33,34] Thus, modified protocols for long term cell culture in channels often need to be developed and require customization based on the cell type. Another issue is the PDMS material used to produce these devices; PDMS has many great attributes that make it the material of choice for fabricating microfluidic devices such as transparency, gas permeability, deformability and easy and inexpensive molding with sub-micrometer resolution.[27] However, several drawbacks are associated with the use of this polymer such as its tendency to absorb small molecules,[35] which could potentially change the concentration of reagents and alter experimental outcome. In addition, researchers showed that the un-crosslinked low molecular weight PDMS leak into the medium and interact with cultured cells.[36]

Recently, we developed novel microfluidic systems that can be integrated with the conventional cell culture protocols (Fig. 1(c)) to take advantage of the strengths of microfluidic systems while avoiding their drawbacks that are associated with the closed-channel configurations. These systems combine properties of microfluidics and of non-contact scanning probes.[31]

2. The Microfluidic Probe

The MFP[31] avoids several limitations that exist with closed-channel microfluidic systems which were discussed above. Typically, the MFP is placed above a substrate to form a narrow gap while immersed within the surrounding liquid (Fig. 1(c)). The gap between the MFP and the substrate is typically in the range of 1 μm to 100 μm. The MFP consists of a flat tip with a pair of apertures, fluidic ports, which are located within a few tens of micrometers of one another. One of the apertures is used to inject a specific solution, while the other is used for aspiration (Fig. 2(a)).

When the aspiration flow rate (Q_A) is considerably higher than the injection flow rate (Q_I), the injected solution is confined and focused by the concentric flow field and consequently deflected into a microjet that is aspirated entirely by the aspiration aperture. Thus, the microjet injected into the gap is confined by the hydrodynamic

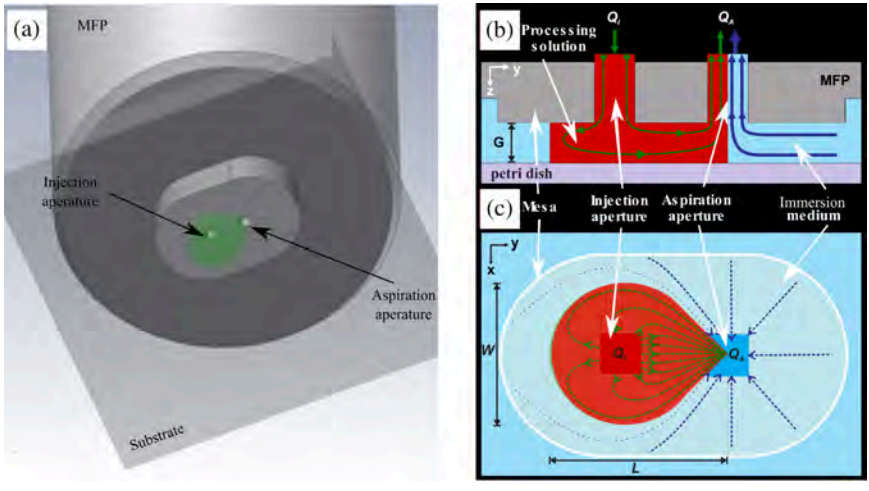

Fig. 2. Schematics of the microfluidic probe. (a) 3D representation of the MFP concept and setup. The injected solution is shown in green. The immersion medium is not shown. (b) Cross-sectional view and (c) bottom view of the MFP showing the push-pull working concept and the processing solution confinement (shown in red). The confinement of the processing solution by the immersion liquid is called hydrodynamic flow confinement. G is the gap between the MFP surface and the bottom substrate. Q_I and Q_A are the injection and aspiration flow rates, respectively. L and W are the length and the width of the tear shape of the confined injected liquid, respectively. (Reproduced with permission of The Royal Society of Chemistry.[3])

forces of the surrounding liquid in a push–pull configuration, called the hydrodynamic confinement, and therefore replaces the solid walls of closed microchannels (Fig. 2(b)). To retain the hydrodynamic confinement, the ratio Q_A/Q_I should remain higher than a certain threshold, otherwise the injected solution leaks into the immersion medium. Using the MFP, the solution microjet can be directly flushed across a sample of interest, which provides a precise tool for localized delivery of reagents. Thus, the MFP prevents the enclosure of samples in closed microfluidic networks; instead, samples can be cultivated using conventional methods (i.e. Petri dishes) and thereby eliminates the need of developing new culture protocols. At the time of experimentation, the MFP approaches the sample from above and delivers reagents to specified areas (Fig. 2(a)). In other words, the MFP defines a mobile channel-less microfluidic device. The MFP therefore can be

used to process large surfaces by scanning across them. The tear shape of the hydrodynamic confinement and its size (L & W) can be tuned by controlling the gap (G) between the MFP and the sample and the ratio of the aspiration to the injection flow rates (Q_A/Q_I) (Fig. 2(c)).

The first developed MFP was made out of a microstructured Si tip which is composed of microfluidic apertures for injecting and aspiratitvng different fluids, and a PDMS block that is bonded to the Si chip.[31,32] The PDMS block in the MFP assembly serves as the world-to-chip interface, and is structured with receiving ports to insert capillaries connected to independent external syringe pumps. The MFP is secured inside a MFP holder for handling and connecting to an XYZ positioning system (Fig. 3(a)).

Several MFPs have been fabricated in different shapes and out of various materials. For example, we designed and fabricated a MFP out of a glass chip that is structured with fluidic apertures, and bonded to a PDMS block to serve as the world-to-chip interface.[37] Another MFP was completely made out of PDMS (Fig. 3(b)) for imaging purposes.[38] Glass and PDMS are transparent and allow for sample illumination through the MFP itself. Moreover, fabricating the MFP out of PDMS using multilayer soft-lithography techniques is inexpensive and less complicated than those made out of Si and glass, and made it easier to fabricate an array of apertures. Connecting different apertures to different reagents would permit for multiplexing and sequential/simultaneous application of different reagents on the same sample.

As various shapes and materials are used for different MFPs, different holders have been produced[32,37,38] to provide low dead volume interface assembly and a simple world-to-chip interface.

An automated setup[32] was developed to precisely control the injection and aspiration flow rates, the parallelism of the MFP surface with the bottom substrate, and the gap between the MFP and the bottom substrate. The setup comprises an inverted microscope that enables live imaging, a probe holder connected to a precise computer-controlled XYZ-stage (Probe Manipulation System), high-precision syringe pumps, and an environmental chamber to control the temperature, humidity and CO_2 levels (Fig. 4). The syringes, which are connected to the computer-controlled syringe pumps, are connected to the MFP via capillaries.

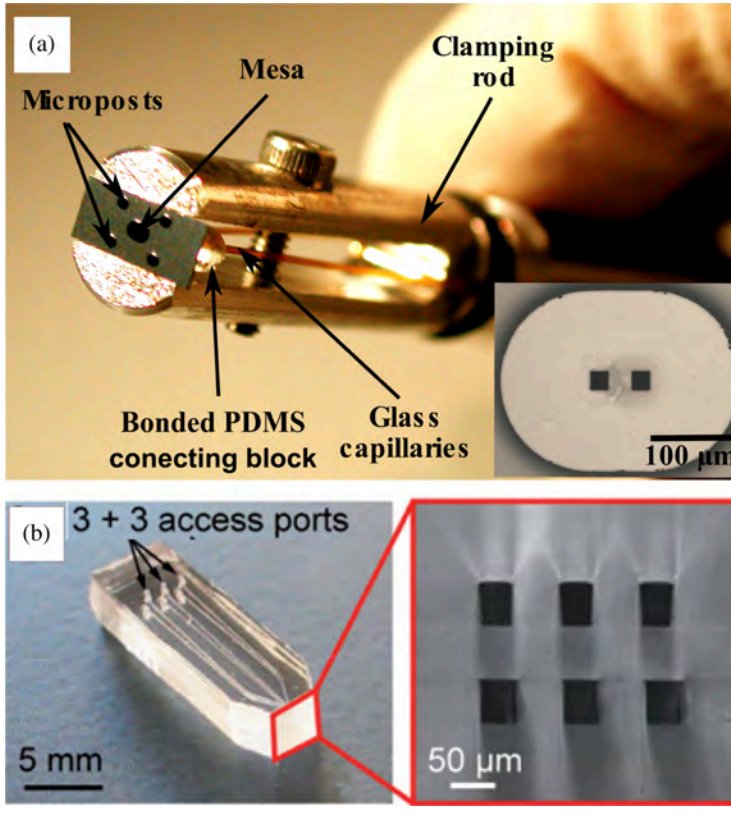

Fig. 3. Different designs and materials for making MFPs. (a) A photograph of the first developed MFP shown while clamped in the holder to connect with the micropositioning system. An enlarged view of the MFP tip and apertures is shown in the inset. This MFP was made out of a Si chip that was bonded to a PDMS block for input/output fluidic connections. The Si chip is etched on both sides to have microfluidic channels on one side, while having a tip shape (called mesa) on the other side. The mesa has two apertures and is surrounded by four microposts which facilitates the parallel alignment of the mesa to the bottom substrate. (b) A photograph of the PDMS-MFP. This MFP was fabricated by laminating three PDMS layers. The two outer layers are patterned using soft-lithography techniques, while the middle layer is a thin flat polymer sheet to separate the apertures from the two outer PDMS layers. This particular MFP has six apertures that could be connected to independent reagents sources for multiplexing. An enlarged view of the tip is shown on the right. (Reproduced with permission from The Royal Society of Chemistry.[3])

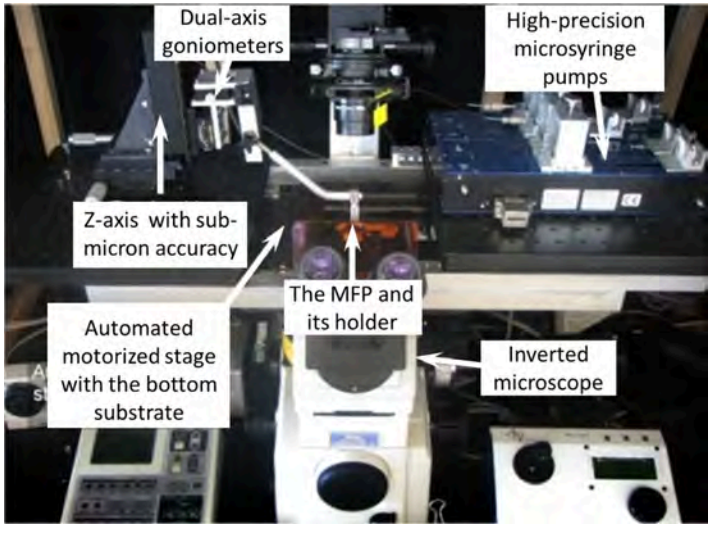

Fig. 4. The developed MFP station. The various components of the station are built around an inverted microscope to permit live imaging during experiments. The Environmental chamber is not shown.

3. Microfluidic Probes to Pattern Surfaces

Patterning surfaces, generally speaking, have wide applications in immunoassays, biosensors, cell biology, drug discovery, and tissue engineering.[39] There are vast methods and technologies to process and pattern surfaces. Notably, robotic inkjet printing and photolithography techniques[40] have been intensively used for processing surfaces. Even though inkjet printing has been the method of choice for microarray patterning, this approach entails some difficulties and challenges. For example, printing is performed in a dry environment, and consequently evaporation and drying of reagents is a major challenge,[40] which leads to patterns obtained with limited control. In addition, complex protein patterns are an issue with inkjet printing as it is mostly restricted to patterning spots on surfaces to form microarrays. On the other hand, photolithographic technologies are highly developed for patterning surfaces and have been frequently used for patterning proteins and cells.[41] Complex patterns can be generated using these methods[42]; nonetheless, several biological samples are not compatible with the typical conditions associated with photolithography, which limits

the use of these methods with some sample. In addition, the high associated costs with its protocols and equipment renders it more challenging for biologists to adopt to and to employ.

Soft lithography, however, has allowed for more flexible and low-cost methods for tailoring and patterning surfaces. Mainly, microcontact printing[43] and patterning using microfluidic channels[43,44] are the most commonly used soft lithography methods for patterning surfaces. These techniques contributed drastically to the field; however, they are inadequate in terms of pattern-on-demand flexibility. After the device is designed and fabricated, it is limited to a single kind of pattern. If another — or a modified — pattern is desired, a new device needs to be designed and fabricated. In addition, issues of world-to-chip interface, complex flow systems, complications in obtaining combinatorial patterns on the same substrate, the nature of direct contact between the substrate and the device and the difficulties of processing large areas are limiting factors of the current soft lithography methods for patterning surfaces.[45] More convenient and on-demand patterning methods are required.

As the concept of the MFP is to remain functional while being immersed in a surrounding liquid, patterning surfaces with biological samples via the MFP overcomes several drawbacks associated with the previously mentioned techniques. Notably, the problems of reagent evaporation, drying, and protein denaturation are eliminated given that the biological samples are processed under physiological conditions. Likewise, because the MFP works in a non-contact mode and is thus mobile; large areas can be processed by scanning the MFP across them. Moreover, due to the small working gap between the MFP and the surface, enhanced mass transfer and molecular adsorption to the surface occur, which results in rapid patterning, low sample consumption, and high spot uniformity.[31] The single spot shape can be altered "on demand" by controlling the hydrodynamic confinement (Figs. 2(b) and 2(c)). Furthermore, complex and advanced pattern shapes can be achieved by controlling the MFP's scanning velocity and direction with respect to the jet flow direction, and the MFP's residency time on spots (the time the probe remains static).

As shown in Fig. 5(a), an array of two different proteins was patterned on a glass slide using a single MFP, with a density as high as 15,000 spots/cm^2. The MFP was programmed to scan across the

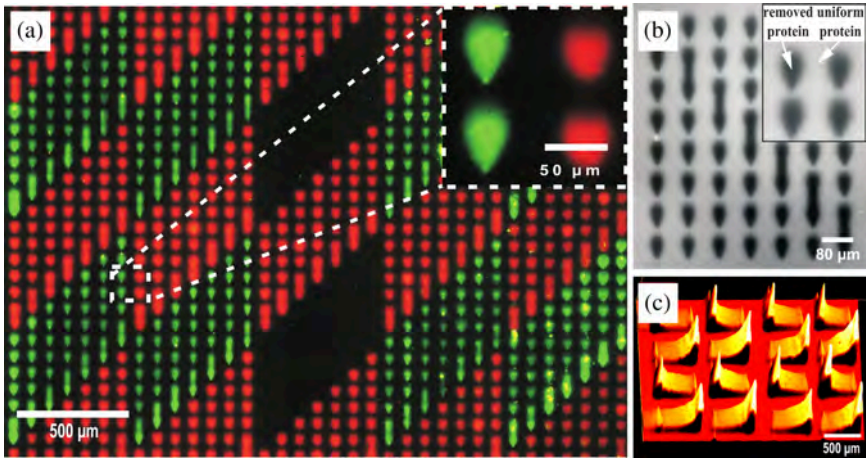

Fig. 5. MFP for advanced patterning of surfaces. (a) Fluorescence micrograph of an array of two different proteins patterned using the MFP. The first deposited protein was a TRITC-labeled goat IgG (red). The second protein, a rabbit IgG, was stained with a FITC-labeled anti-rabbit IgG (green) following the patterning process. (b) Fluorescence micrograph of an array of TRITC-labeled goat IgG achieved by subtractive patterning. The white color represents the homogenously adsorbed protein on the glass slide from solution, and the black spots represent the local removal of the IgG using the MFP by delivering a stream of solvent solution. (c) An intensity profile of the patterned array of TRITC-labeled goat IgGs gradient islands on a glass slide. The intensity clearly illustrates the achieved surface-density protein gradients obtained by ramping the MFP's scanning speed. (Reproduced with permission from The Royal Society of Chemistry.[3])

substrate. Instead of obtaining isolated spots by stopping the flow, they were achieved by the simple stop-and-go movement of the MFP. The residency time was 0.3 s for this specific case. The array of the first protein was performed initially, and subsequently the injection aperture was connected to a second syringe containing another protein solution that was deposited using the same procedure on the same substrate to obtain an array of two different proteins on a common substrate. To demonstrate the ability to control the single spot shape, we connected the last two spots of each column of each of the two proteins (making a protein segment), simply by scanning the MFP back-and-forth. Moreover, a unique feature of patterning using the MFP is subtractive patterning, whereby proteins were uniformly deposited on a glass slide and then selectively dissolved using the

MFP to achieve the negative of the previously mentioned patterns (Fig. 5(b)). Once more, the segment patterns were obtained by the back-and-forth scanning motion of the MFP.

More advanced patterns can be generated with the MFP. For example, by programming the MFP to continuously change the scanning speed from one point to another, we were able to control the amount of deposited protein on a glass substrate. By gradually decreasing the scanning speed, we successfully patterned protein gradients (Fig. 5(c)). Several distinct gradient islands were achieved through the rapid scan of the MFP while moving from one island to the next, which prevented the protein absorption within these regions of separation.

4. Microfluidic Probes to Alter Cellular Microenvironments

As we discussed in the previous section, there are many alternative possibilities that the MFP technology can bring to the field of surface patterning. In this section, we are describing how the MFP technology can be used to manipulate and label cells *in vitro* selectively, and how it can control the cellular microenvironment of the cell culture.

Conventionally, pipetting is one of the major steps used in experiments involving *in vitro* cell cultures. Drugs are typically pipetted into cell containers and eventually a uniform stimulation of the entire population of cells occurs. This state does not mimic the real *in vivo* processes, where the microenvironment is different from point to point and cell to cell. The local microenvironment is tightly controlled and regulated by the extracellular matrix molecules, soluble factors, and neighboring cells.[46] Moreover, equipment and user errors are main caveats in the procedure of pipetting. Microfluidics, however, is controllable and repeatable,[5] and allows for local and partial stimulation of cells,[47,48] which opens a new avenue to study cells under spatiotemporally-controlled stimulations. These techniques would greatly facilitate the design and achievement of novel and precise experimentation to better understand *in vivo* biology. Nonetheless, microfluidic devices suffer the closed-channel configuration which leads to several drawbacks as we described earlier in this chapter.

The MFP technology, conversely, is well suited to selectively controll cellular microenvironments at the subpopulation and subcellular levels. For example, selected cells in a culture can be detached, added, or moved to a new location, without affecting neighboring cells. As shown in Fig. 6, the MFP was placed atop of a cell culture in a dish filled with cell medium. The MFP was then moved above a selected cell, and used to locally flush the cell with a solution of Trypsin (Fig. 6(c)). A few minutes later, the selected cell was detached from the culture and dragged into the aspiration aperture (Fig. 6(d)). The aspiration flow could be switched to injection, and the cell could then be retrieved and deposited in a new location on the same or a different dish. This way, the MFP could be used as a tool to move cells around, and could be envisioned as a microfluidic tweezers. Indeed, adding or removing selected cells changes the local microenvironment of a cell population, and could

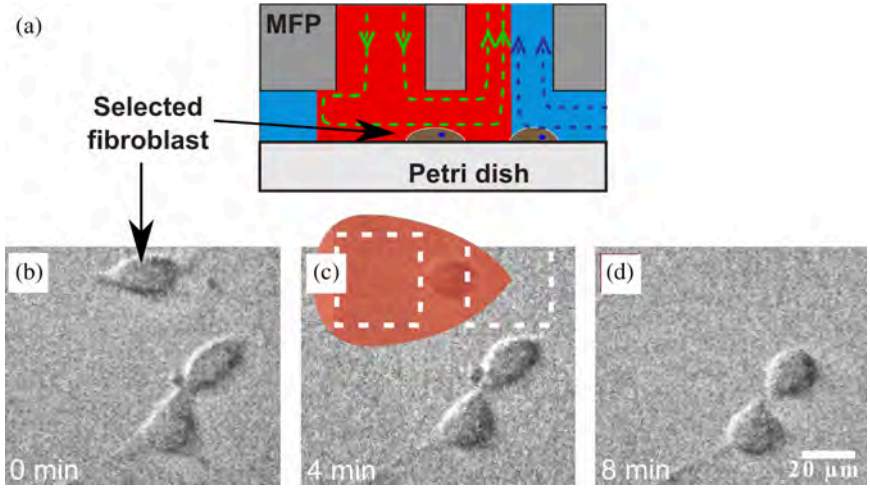

Fig. 6. The MFP is used to detach and collect selected living adherent cells. (a) A cross-sectional schematic of the MFP while placed atop a selected cell in the cell culture. (b) A micrograph showing the selected and two neighboring fibroblast cells. (c) Schematics are added to the cell micrograph to outline the position of the MFP apertures and the localized Trypsin microjet (red). (d) The selected cell was detached from the cell culture while neighboring cells remained unaffected. (Reproduced with permission from The Royal Society of Chemistry.[3])

empower many interesting studies in cell biology, such as research on cell–cell interactions.[49]

Moreover, the MFP can be used to selectively label subpopulation of cells in a dish (Fig. 7(a)). The process is similar to writing on a monolayer of cultured cells without any contact, while leaving other surrounding cells unlabeled. Similarly, living cells can be targeted and selected as single entities or subpopulations of cells to be labeled, Fig. 7(b), or even deactivated, Fig. 7(c). Using the same procedure, subpopulation of cells could be deactivated and then removed from the dish altogether, for the study of cellular migration, cellular division, or tissue reorganization.[50,51]

The MFP is well suited for the study of cellular dynamics, where selected cells — or a part of a cell — can be locally perfused with chemicals for several hours while being imaged to observe cellular dynamics. With the MFP, individual cells in the dish can be selected and the chemical perfusion can be precisely delivered to a chosen part of the cell. This flexibility and specificity is nonexistent in the closed-channel microfluidic system approach, where only part of the cell population is accessible.[47,48] More importantly, the open microfluidic configuration of the MFP brings the possibility of coupling it with other technologies and protocols at the time of the experiment. These features of the MFP make it an attractive tool for research in neuroscience, for example, where neurons are highly branched cells that connect to each other and form networks. For example, an axon from a fixed neuronal culture was targeted and selectively perfused with a drug that is taken up by the myelin sheaths surrounding the axon (Fig. 7(c)). This experiment will be extended to perfuse axonal bundles that were grown in culture for several months[52] to study of myelination and discover drugs that promote axonal insulation. Another example is the use of the MFP for the study of synaptic plasticity in a network of hippocampal neurons. A few axons were perfused with a drug to investigate the dynamics of synaptic receptors on the cell membrane (Fig. 7(d)). This experiment can be coupled with microelectrode array technology[53] for the local electrical stimulation/measurement of the neuronal activity.

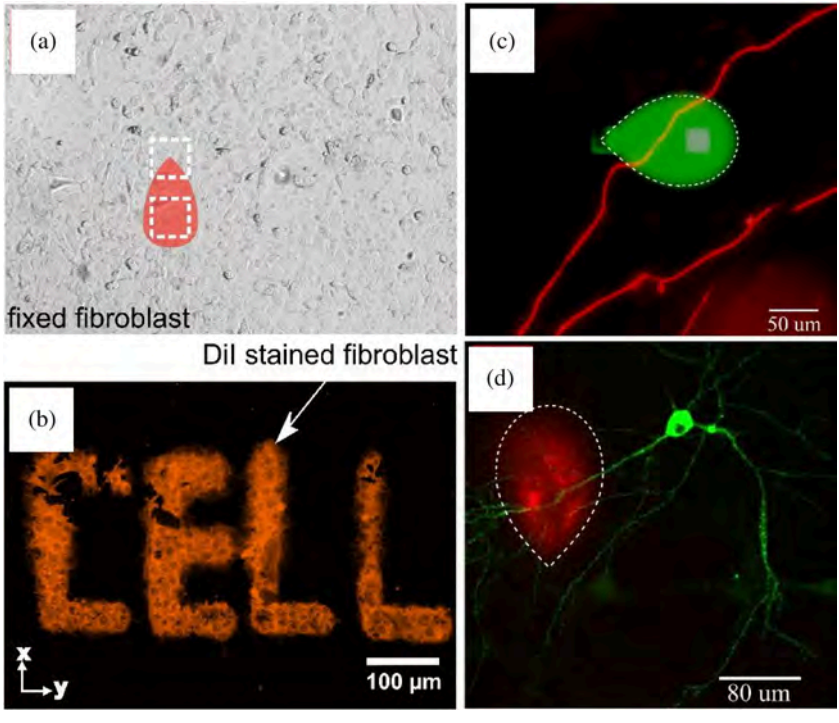

Fig. 7. MEPs to selectively label cells and to study cellular dynamics. (a)–(b) A fixed monolayer of fibroblasts was processed selectively with a MFP so that only selected cells were labeled with a fluorescent dye (DiI) to have the word "CELL" geometery, while others are left untouched. (a) An optical image of the cell monolayer with schematics showing the locations of the apertures and the microjet boundaries. (b) The final fluorescent image highlighting the labeled and unlabeled cells. (c) Local perfusion of a selected axon in a fixed culture of dorsal root ganglia neurons in a dish. Neurons were stained with FluoroMyelin (red) to label myelin. The MFP was perfusing a solution of Fluorescein (green) to demonstrate the local stimulation. (d) Stimulation of a selected dendrite of live neuronal network (rat hippocampal neurons) with tumor necrosis factor-alpha (TNF) for the studies of synaptic plasticity. Neurons are transfected with pHluorin-GluR1 (green) which labels GluR1 receptors. TNF is mixed with dextran (red) to visualize the local stimulation. (Reproduced with permission of The Royal Society of Chemistry.[3])

5. Microfluidic Probes for Local Processing of Tissue Slices

Laboratory research based on 2D *in vitro* cultures of dissociated cells is of significant value because of its contribution in shaping our current understanding of *in vivo* biology. Similarly, there is an elevated interest in studying tissue slices that are obtained through biopsies.[54]

Tissue slices, a 3D complex network of various cell types that possess the natural tissue-matrix configuration, represent the actual physiological settings of the organ far beyond those present in *in vitro* cultures of dissociated cells.[55] Thus, for a variety of studies, i.e. those applied in the pharmaceutical industry, there is high demand for experimentation on actual tissue slices.[56,57] For example, preclinical studies of new drug candidates on human tissue slices would be more relevant to the real *in vivo* systems than those achieved by studying the 2D cell-culture or animal models. Using tissue slices, information about metabolism and toxicity of the candidate drug can be obtained in an environment that better mimics the organ response *in vivo*.[58] Conventional methods for culturing and handling tissue slices are complicated by such factors as the lack of ability to control the tissue's microenvironment, the labor-intensive methods and the low throughput read out. Conversely, microfluidic technology can be used for studying tissue slices[59] and offers a diminished reagent consumption, multiplexing capabilities and an increased result throughput.[60] Microfluidics facilitates the spatiotemporal controllability of the tissue microenvironment and also allows for long-term tissue culture with continuous laminar perfusion.

Several microfluidic devices were developed to culture tissue slices and are used in a variety of experiments, such as for electrophysiological studies,[61] biomarker discoveries,[62] and toxicology studies.[63] Nonetheless, the use of microfluidic technology for tissue slice applications suffers from closed channels configurations. It is even more challenging to controllably place a large tissue slice inside a closed chamber than it is to seed dissociated cells in this setup. This limitation is the main reason for which the application of microfluidic devices for tissue slices has remained sparse in comparison to

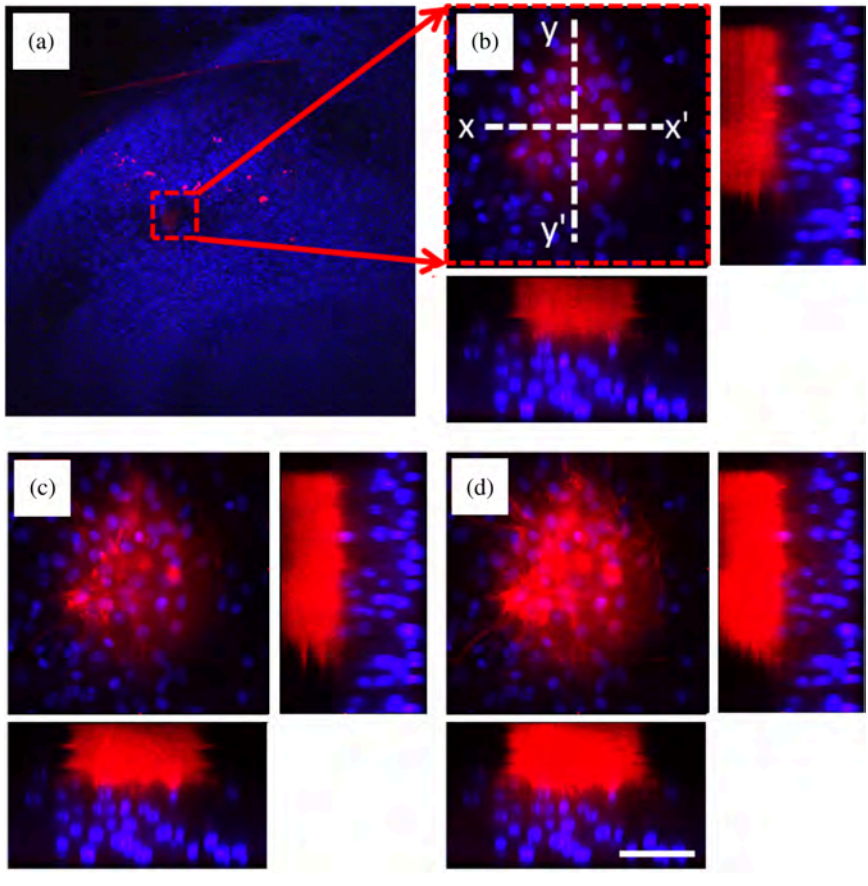

Fig. 8. A fixed organotypic slice from an L15 transgenic mouse was perfused locally with dextran for 19 min using a MFP. (a) An overview of a fixed and stained mouse organotypic hippocampal slice. The blue (DAPI) color represents the cell nuceli. The slice is placed in a chamber that has a coverslip on the bottom to permit high resloution microscopy. (b)–(d) Time-lapse confocal images of the dextran (red) distribution at a depth of 32 μm inside the 70 μm thick slice. The main micrographs show the XY dextran distribution at 2 min (b), 10 min (c), and 19 min (d), following the perfusion. The insets are showing the YZ and XZ cross-sectional views of the slice and the dextran penetration through the slice. The scale bar is 40 μm.

widespread cell culture applications. A number of open microfluidic chambers have been developed for culturing and perfusing tissue slices,[63–65] however, closed channels or more complicated features and designs are needed when experiments require controlled local stimulation[66] and multiplexing.[60]

On the contrary, MFP technology works in open systems and is well suited for local stimulation, thus eliminating the need to cultivate tissue slices inside closed chambers. Instead, tissue slices are cultured in open perfusion systems where the MFP is applied. Having the open configuration, the MFP local stimulation can be coupled with other technologies at the time of experimentation, such as live microscopy imagining and electrophysiology experiments. This flexibility of the MFP technology renders it an ideal platform for tissue-based biological studies.

In order to demonstrate the functionality of MFPs with tissue slices, we used the MFP to locally perfuse an organotypic hippocampal brain slice with a fluorescent dye.[38] Organotypic brain slices mimic the brain's *in vivo* morphology, neuronal connectivity and synaptic transmission, and demonstrate preserved glial-neuronal complexes.[67] Organotypic brain slices have therefore been used to study learning and memory, neuronal regeneration following injuries, and they also have been used as disease models such as for epilepsy.[68] We placed the fixed organotypic hippocampal brain slice on a perfusion chamber that was designed to fit in an inverted confocal microscope for imaging. The MFP was then introduced from the top to locally perfuse fluorescent dextran (red). Using live confocal imaging, we studied the distribution of the dextran perfusion on the slice and its penetration in terms of depth within the slice (Fig. 8). Results showed that the dextran penetrated to a depth of 32 μm inside the 70 μm thick slice after 19 min of perfusion. Obviously, this experiment highlights the potential of using the MFP for local perfusion on live tissue slices, and could even be empowered by integrating other technologies such as two-photon fluorescence imaging.

6. Conclusion and Outlook

Throughout the different sections of this chapter, we discussed several benefits that microfluidic devices can bring in comparison to traditional methods used in biology laboratories and the biotechnology industry. We highlighted the associated limitations and drawbacks of the close-channel microfluidic devices and explained how the MFP technology can overcome them. The MFP technology is an open microfluidic system, which takes advantage of the controllable and predicable flow of fluids at the microscale, but also integrates the features of scanning probes by providing mobility in a non-contact nature. The principle of the hydrodynamic confinement was achieved by having the MFP form a narrow gap with the bottom substrate and maintaining an aspiration to injection flow ratio higher than a certain threshold value. Thus, the aspiration flow created a concentric flow that was strong enough to deflect and confine the whole injected solution without any physical walls. The hydrodynamic confinement can be controlled and localized precisely by controlling the flow rates and the gap between the MFP and the bottom substrate. In the future, internal pumps and other components such as proximity sensors could be integrated within the MFP itself to minimize the setup footprint and enhance MFP precision. We further introduced the different MFPs that were developed, which vary based on their designs and materials. We expect to see other new designs and materials defining the MFPs in the future, where depending on the experimental conditions, some designs and materials would be advantageous over others. For example, we recently developed a novel MFP with four apertures to generate concentration gradients within a quadrupolar fluid flow.[69]

The MFP technology's applicability is not limited to biology, but could also find applications in surface chemistry, polymer electronics and microfabrication. However, we focused this chapter on the MFP's biological application to remain focused on the general theme of the book. We showed how the MFP can be used to pattern proteins on surfaces with multiplexing ability. The patterning shape can be configured on demand, and complex shapes can be generated

by controlling the scanning speed and direction. Problems of evaporation and denaturation were eliminated using the MFP. Furthermore, we showed how the MFP can be used to manipulate individual adherent cells, label single cells or subpopulations of cells and how it can be used for localized chemical stimulation at the subcellular level. There is no need to cultivate the biological samples inside channels or to develop new culturing protocols when using the MFP; the MFP works with the conventional protocols. We hypothesize that MFP technology will be of great use in studying sensitive cells such as primary neurons or stems cells.

We explained how the MFP technology suits large biological samples and can be used with tissue slices or embryos, for example. Using the MFP, tissue slices were perfused locally and in a selective manner. This way, local stimulation of tissue slices can be coupled with other techniques such as electrophysiology electrodes.

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