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Microfluidics

Microfluidics in the "Open Space" for Performing Localized Chemistry on Biological Interfaces

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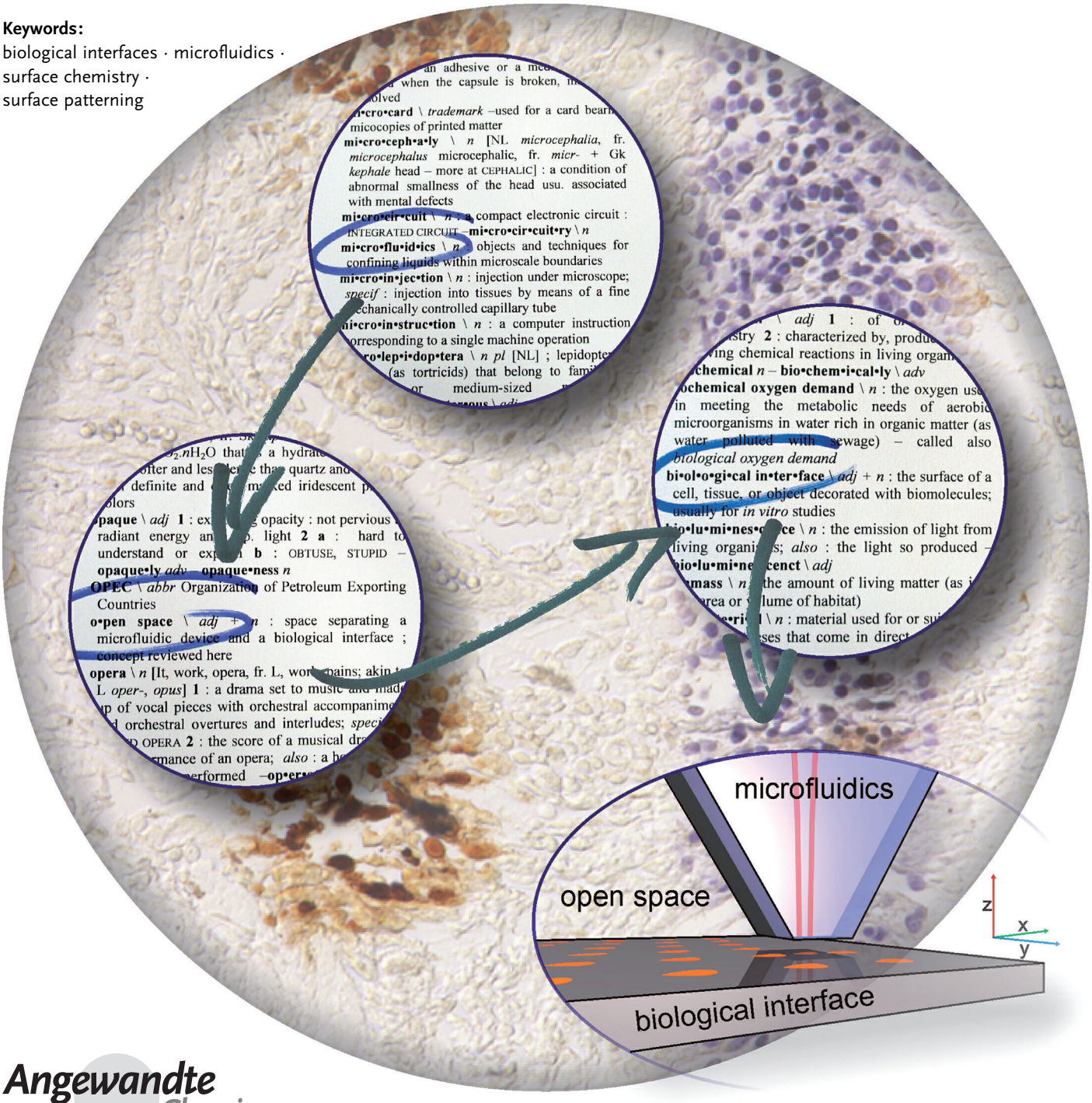

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Local interactions between (bio)chemicals and biological interfaces play an important role in fields ranging from surface patterning to cell toxicology. These interactions can be studied using microfluidic systems that operate in the "open space", that is, without the need for the sealed channels and chambers commonly used in microfluidics. This emerging class of techniques localizes chemical reactions on biological interfaces or specimens without imposing significant "constraints" on samples, such as encapsulation, pre-processing steps, or the need for scaffolds. They therefore provide new opportunities for handling, analyzing, and interacting with biological samples. The motivation for performing localized chemistry is discussed, as are the requirements imposed on localization techniques. Three classes of microfluidic systems operating in the open space, based on microelectrochemistry, multiphase transport, and hydrodynamic flow confinement of liquids are presented.

From the Contents

1. Introduction

Microtechnology, microfluidics, and soft lithography have provided the main steps in linking technology to biology and life sciences in general. These techniques are broadly explored by microtechnologists and chemists, and used by biochemists and biologists for protein patterning, assay miniaturization, diagnostics, advanced purification, and separation.[1] The next critical step is to increase the compatibility of microfluidics with biological systems that are sensitive to the environment (such as, fragile proteins on surfaces, living cells). In addition, they should also be compatible with glass slides and Petri dishes. Moreover, as living matter "likes" surfaces, substrates that are functionalized for biological applications are increasingly used and commercially available. Microfluidics should be able to interact with such substrates in the "open space", which will facilitate the study of biological samples. To succeed in these endeavors, microfluidics needs to eliminate one of its major constraints: the walls.

Numerous applications require control of chemical and biochemical reactions on the micro- to nanoscale on biological interfaces. For example, interaction with adherent cells is highly desirable in biomedical research for engineering cellular architectures, modulating stem-cell microenvironments for regenerative medicine, presenting gradients of guidance molecules for understanding processes in cell and developmental biology, and dispensing chemicals at various concentrations for drug screening and toxicology studies.[2–5] The volumes and length scales in microfluidics are generally compatible with those in the life sciences, but also the inherent components of this technology must be biocompatible.

There is now a greater need than ever before for developing technologies[6] that could become just as ubiquitous in life-science laboratories, as the ever-present microscopes and micropipettes. In addition to precisely outlining the need and specification of a technology, driven by a biological/clinical investigation, careful considerations of the physical and chemical mechanisms underlying microfluidics are required. For example, widely used assays, such as DNA/protein microarrays and immunoassays, are based on analyte–receptor interactions, and understanding these interactions allows the assay designs to be improved further. Therefore, the key to a successful implementation of tools for the localization of chemical functions or reactivity on biological interfaces is an in-depth knowledge of the interplay between diffusion, convection, and reaction,[7] and of how a system will behave based on the fluxes, equilibration times, and fundamental quantities of the chemical species involved.[8]

1.1. Scope

In this Review we first outline the need and motivation for performing localized chemistry on biological interfaces and the requirements imposed on localization techniques. A snapshot of the present microfluidic implementations for handling biological specimens within closed-systems is then presented. The transition from closed to open microfluidic systems is outlined, starting from the removal of one wall ("the lid"), thereby making the system partially open. Finally we describe three classes of microfluidic systems operating in the open space based on microelectrochemistry, multiphase transport, and hydrodynamic flow confinement of liquids.

We bias this Review towards biological systems and interfaces because we believe that this is where microfluidic technology is most needed to solve important problems in healthcare and life sciences and where it can have a broad impact.

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1.2. Why Localize Interactions with Biological Surfaces in the "Open Space"?

A fundamental characteristic of biology is compartmentalization. Biochemically speaking, reactions should occur at the right place and at the right time in cells and tissues. For this reason, most approaches for studying and transforming cells are based either on encapsulating liquids (e.g. nanodroplets, vesicles) or on engineering physical compartments for cells (e.g. scaffolds, microfabricated chambers). However, both approaches are restrictive. An excellent example illustrating the idea of spatial and temporal sequencing of biological events is described by Healy.[9] During embryonic development, different cell populations contribute to the overall organization of the tissue, and chemical signaling within the developing tissue and organs controls the fate of cells. Biologists have long attempted to capture these processes outside of the body. Ex vivo methods for studying cell biology have contributed to the vast knowledge of mammalian cell function, but the spatial and temporal presentation of soluble and physical signals to cells continues to be difficult.[9]

Techniques offering the ability to study, work, stimulate, and locally interact with adherent cells and tissues in an "open space" would yield breakthroughs in understanding the biology and physics of biological interfaces, similar to the advancement of our knowledge enabled by other techniques. Patch-clamp, for example, is a well-established technique[10] that relies on the pressure-based contacting of cells with a 1–2 μm diameter micropipette. It is widely used for measurements of the membrane potential and has provided significant insights into cell functions. Surface-based assays are inherently performed without total encapsulation (e.g. microtiter plates and glass slides), and there is a trend to use microfluidics to enhance their performance. We therefore believe that the power of microfluidics can be leveraged when implemented for use in the "open" space, without physical contact or constraints, allowing the interaction with surface assays and biological interfaces. Figure 1 aims to capture the set of emerging techniques for the local processing of biological interfaces and their applications in biology. Several of these examples are applicable in cell biology and tissue research. The scope of this Review is not restricted to cellular analysis, and deals with localization on biological interfaces in general.

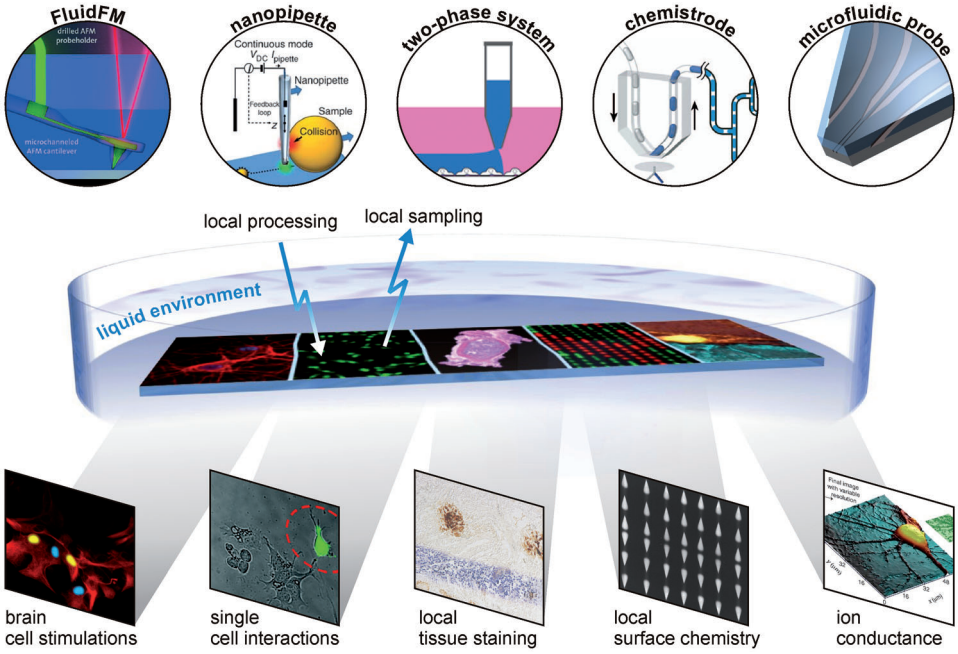

Figure 1. Emerging methods for performing (bio)chemical processes locally on biological interfaces in liquid environments. Sources of images are listed in subsequent Figures.

1.3. Microfluidic Technology for Localized Chemistry

Scientists developing microfluidics initially chose the path of using closed microfluidic channels and chambers. For specialized applications, such as in immunodiagnostics, closed-channel microfluidic devices have widely been used.[11] However, the high hydraulic resistance, the difficulty

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Govind Kaigala is at IBM Research, Zurich, where he works on research activities at the intersection of biosciences with micro- and nanotechnologies. He had his postdoctoral training at Stanford University and received both his Ph.D. and M.Eng. from the University of Alberta. His research interests include the design and development of microsystems and assays involving chemical and biomolecular analysis for pertinent medical and clinical applications.

to introduce samples, such as tissues, cells, and mesoscopic objects into microdevices, and the clogging by particulates and air bubbles limit the practical use of closed microfluidic devices. Moreover, biological samples are sensitive to their environment and are prone to drying artifacts and denaturation. Despite significant progress in microfluidic technologies, there is a strong motivation to develop new and improved approaches to control the chemical and physical environments around intact cells in tissue or cell cultures. Glass micropipettes have traditionally been used to alter the cellular environment and to deliver active compounds to biological interfaces, including to single cells. This method is however not suitable for maintaining a localized environment because the active substrate can diffuse outside the field of analysis. Sims and Allriton[12] published a comprehensive Review describing the motivation behind and the microfluidic technologies for single-cell analysis.

It is important to have technologies that can localize liquids on length scales that are commensurate with biology. The origin of liquid localization is inkjet technology, starting from printing ink to patterning biological molecules. In parallel, advances in microtechnology have enabled the fabrication of devices for measuring complex biophysical and biochemical characteristics of cells and sub-cellular components.[13,14] A technology called microcontact printing (μCP),[15,16] now widely used to pattern chemicals on a broad range of length scales on surfaces,[17] is simple enough for use by nonspecialists. Microcontact printing has been applied in various fields, such as in the fabrication of protein biochips[18] and in the preparation of surfaces to understand cell-surface interactions.[19] It involves transferring molecules from a stamp onto a surface. For any modification of the pattern, the stamp has to be redesigned, which is tedious and time consuming.

Broadly speaking, the microfluidic approaches pursued in the context of localization are the following.

Microfluidics for handling cells: A multitude of techniques, such as electrophoresis, dielectrophoresis, electroporation, surface acoustics, and Raman spectroscopy have been miniaturized and integrated into microfluidic devices, some of which exhibit great promise for cell analysis and interaction. However, the use of these closed channel microfluidics still poses many challenges, so that investigations of adherent cells and tissues within a closed device may not reflect the true biology of cells.

Robert D. Lovchik studied biotechnology at the University of Applied Sciences (UAS) in Waedenswil (Switzerland) and received his engineer's degree in 2000. He obtained his M.Sc. in micro- and nanotechnology from the UAS Vorarlberg (Austria) and joined IBM Research, Zurich as a research engineer in 2007. His current work focuses on the microfluidic probe technology and the development of a microfluidic platform for cellular studies. In parallel, he follows a Ph.D. program from the ETH Zurich under the supervision of Prof. Viola Vogel.

Microfluidics for compartmentalization: In the past few years, droplets encapsulating an aqueous phase have provided an even smaller and more powerful form of "compartmentalization" than microtiter plates and microarrays. The volumes of liquid encapsulated in these droplets can accommodate just one or a few cells. They have been used for many applications, ranging from DNA sequencing, biochemistry, and drug screening to directed evolution.[20] One of the techniques based on droplets is electrowetting on dielectrics,[21] also called digital microfluidics.[22,23] There, droplets are moved on a surface by means of an array of electrodes that modify the wetting characteristics of the surface. This technology shows great promise towards open-space microfluidics, but requires engineered surfaces.

Collectively, these microfluidic methods mainly rely on direct contact with a solid interface and do not allow the patterning of delicate surfaces of living cells as physical contact may damage them. Although existing microfluidic implementations of assays have made significant progress towards enabling capabilities otherwise infeasible, they still remain restrictive when dealing with surface-based assays. Some microfluidic implementations of biological assays are described below and shown in Figure 2.

Park et al.[24] developed microfluidics for multiplexed immunohistochemistry (MMIHC) on tissue sections. They used microchannels made in polydimethylsiloxane (PDMS) to draw solutions containing primary antibodies for detecting disease markers in tissue sections, see Figure 2 a. They showed that four biomarkers, namely, estrogen receptor, human epidermal growth factor receptor 2, progesterone receptor, and Ki-67, can be detected in parallel on breast-cancer cells and human breast cancer tissues. Although this approach enables multiplexed IHC, it still is restrictive because the footprint of the microfluidic channels on the tissue section is determined arbitrarily by the design and fabrication of the microfluidic device. Small, damaged, or heterogeneous tissue sections can render this approach challenging.

Along the lines of local IHC, there is merit to develop techniques for multiplexed in situ hybridization assays on tissue sections. Dufva et al.[25] demonstrated online monitoring of hybridization with fluorescein-labeled probes to 18S ribosomal RNA in mouse-brain tissue sections. They called their device HistoFlex. It provided uniform hybridization conditions across the reaction chamber, as determined by hybridization of spotted DNA microarrays. HistoFlex achieves a higher sensitivity than conventional techniques for the detection of miRNAs, but requires significant optimization in advance to account for varying tissue histology.

For visualizing, probing, and manipulating the metabolic and structural machinery of mammalian cells, Whitesides and colleagues[26] developed a microfluidic technique and called this "partial treatment of cells using laminar flows" PARTCELL. They used it to study the subcellular processes of mitochondrial movement and changes in cytoskeletal structure in living cells. The microfluidic systems were prepared by placing PDMS with molded channels on top of a living cell. Parallel streams of different liquids were created in the microfluidic channel, Figure 2 b. The width of each stream and the position of the interface between adjacent streams were

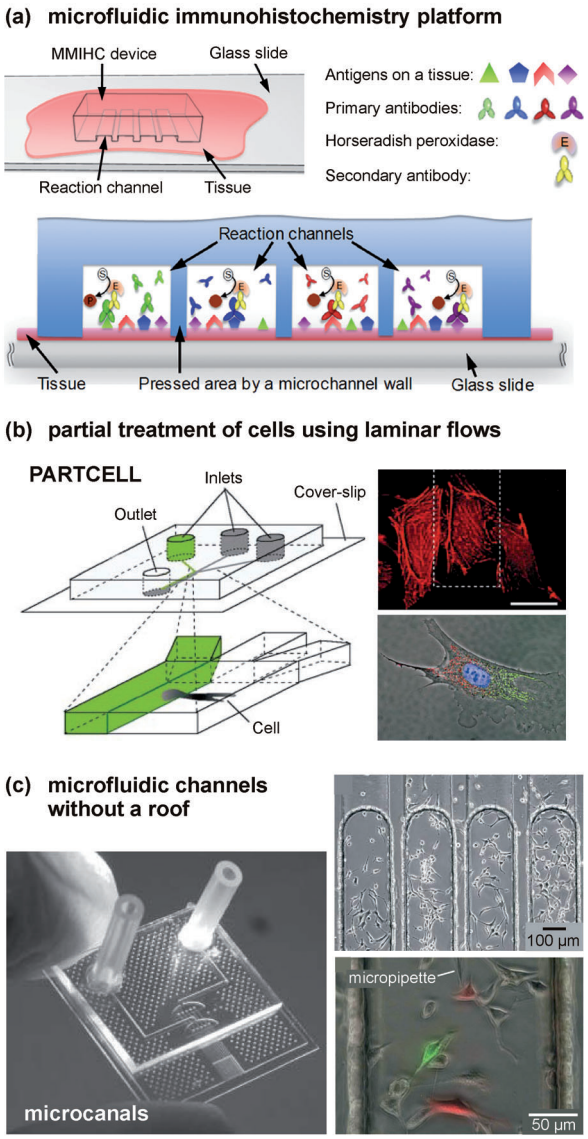

Figure 2. "Closed" channel microfluidics for processing local regions on tissue sections and cells. a) PDMS with channels placed on tissue sections for the delivery of antibodies for local immunohistochemistry. b) Partial treatment of cells using a microfluidic device. c) "Microcanals" made in glass and PDMS with holes for pipetting nutrients/dyes to selected cells. Images reproduced with permission and their source: (a) Reproduced from Ref. [24], copyright 2001 Macmillan Publishers. (b) Reproduced from [26], copyright 2001 Macmillan Publishers. (c) Reproduced from Ref. [27], copyright 2004 Royal Society of Chemistry.

controlled by adjusting the flow rates. The cells were positioned between two adjacent streams, one with and the other without the molecule of interest. Figure 2b shows the disruption of actin filaments in selected cell regions after treatment with latrunculin A, a membrane-permeable molecule that binds to actin monomers. This approach increases the resolution in staining cells by using microfluidic elements with large features that are easy to fabricate. However, much like the device developed by Park et al.,[24] the footprint of the microfluidics needs to be altered for different cell distributions.

Micropipette manipulation and probing of cells within a microfluidic environment can expand the functionality of several existing "closed" channel microfluidics. To this end, Folch et al.[27] developed an open-air microfluidic device without roof. This technique, which they called "microcanals" constituted a cell-culture environment accessible with micropipettes (Figure 2c). The microcanals were fabricated in PDMS and used glass as substrates. Despite being in open channels, the liquid was confined to the microcanals owing to the wetting of the bottom corners and the hydrophobicity of the top surface of the microcanal walls. Cells were seeded in the microcanal floors by adding a cell suspension to the corresponding inlet and stopping the flow during cell attachment/spreading. Patch-clamp electrophysiological recordings from human kidney cells were performed. This approach provided a solution for interacting with the cells seeded on surfaces in semi-closed microchannels, but is only suitable for interacting with a limited number of cells.

The techniques described above are good examples of how cells and tissues can be investigated using microfluidics. The technique based on microcanals is a transition from closed to open microfluidics. By removing one wall, an open space over the biological specimen is created and the sample can be probed directly using patch-clamp technology, which improves the flexibility and interactive capability.

2. Requirements for Performing Localized Chemistry on Biological Interfaces in the "Open Space"

The previous Section demonstrates how closed-channel microfluidics evolved to accommodate tissues and adherent cells for biological studies. More flexible and interactive tools are nevertheless needed to cope with the variability inherent in biological specimens. Only a limited number of techniques exist that are compatible with biological interfaces, particularly for processing in the "open" space. Techniques localizing chemical reactions on biological interfaces should ideally be:

- Non-invasive: they should preferably work in a "non-contact" mode to minimize the perturbation of the biological interface.
- Immersed: the presence of a buffer/liquid environment would prevent drying artifacts, such as denaturation.
- Biocompatible: no toxic materials or chemicals should be needed, and the techniques should be compatible with typical ranges of pH values, temperature, ionic strengths, sheer forces, and pressures.
- Flexible: compatibility with different materials, topographies, length scales (µm to cm), and volumes inherent to biological systems would be beneficial.
- Interactive: the technique should provide feedback (e.g. current, voltage, force, optical signal) during interaction with the biological interface.

When using a partially or a fully open architecture, evaporation is not necessarily a problem, as it is easy and convenient to immerse the sample in larger volumes (mL range) of liquids or to provide an environment with high

relative humidity. The key advantage with the open architecture, is that there is no need to flow cells and tissues through a closed microchannel for processing, instead, the (bio)chemicals are brought to the biological interface.

3. Strategies to Perform Localized Chemistry on Biological Interfaces

Shortly after its invention, scanning probe microscopy (SPM) was utilized to probe surfaces and interact with (bio)molecules on surfaces. With the addition of microfluidic functionalities, various techniques derived from SPM emerged for localizing (bio)chemical processes over biological interfaces. Interestingly, these techniques meet a fair number of the requirements listed in Section 2 for localizing chemical reactions on biological interfaces. We therefore Review these techniques next, before we discuss microfluidic demonstrations based on microelectrochemistry, multiphase transport, and hydrodynamic flow confinement of liquids, which meet even more of the above requirements.

3.1. From Scanning Probe Microscopy to Scanning Techniques having Microfluidic Features

Scanning probe microscopy[28] covers a set of techniques having high positioning accuracy that are used in material sciences, lithography, metrology, and probing biomolecules on surfaces. In these techniques, a probe is moved line by line to record the probe–surface interaction as a function of the position. Among these techniques, the atomic force microscope (AFM) is the best known and most used. It uses a cantilever to probe surface properties, such as the topography, elasticity, electric potential, magnetism, and mechanical resistance. Originally developed for investigating the physics of materials, the AFM soon started to be used to address some of the pressing needs in the life sciences, such as the precise manipulation and interaction with biological samples. A key extension to AFM and related techniques was the recent inclusion of fluid-handling/dispensing capabilities, building a bridge between the SPM world and biological sciences.

The Mirkin group used AFM cantilevers to pattern a range of biomolecules on surfaces and called the technique dip pen nanolithography (DPN).[29,30] Originally, DPN was used to transfer alkane thiolates from a cantilever to a gold surface in air, enabling self-assembled monolayers (SAMs) on gold to be patterned with sub-100 nm resolution. The DPN cantilever is coated with a chemical ("ink") and transfers it locally to the surface by contact and diffusion (Figure 3a). Cantilevers are made from silicon nitride and functionalized to increase the hydrophobicity of the tip. The liquid deposition properties are determined by the interactions between ink and tip, ink and surface, and the properties of the ink, such as surface tension, viscosity, and vapor pressure under the writing conditions. AFM-based approaches work in non-

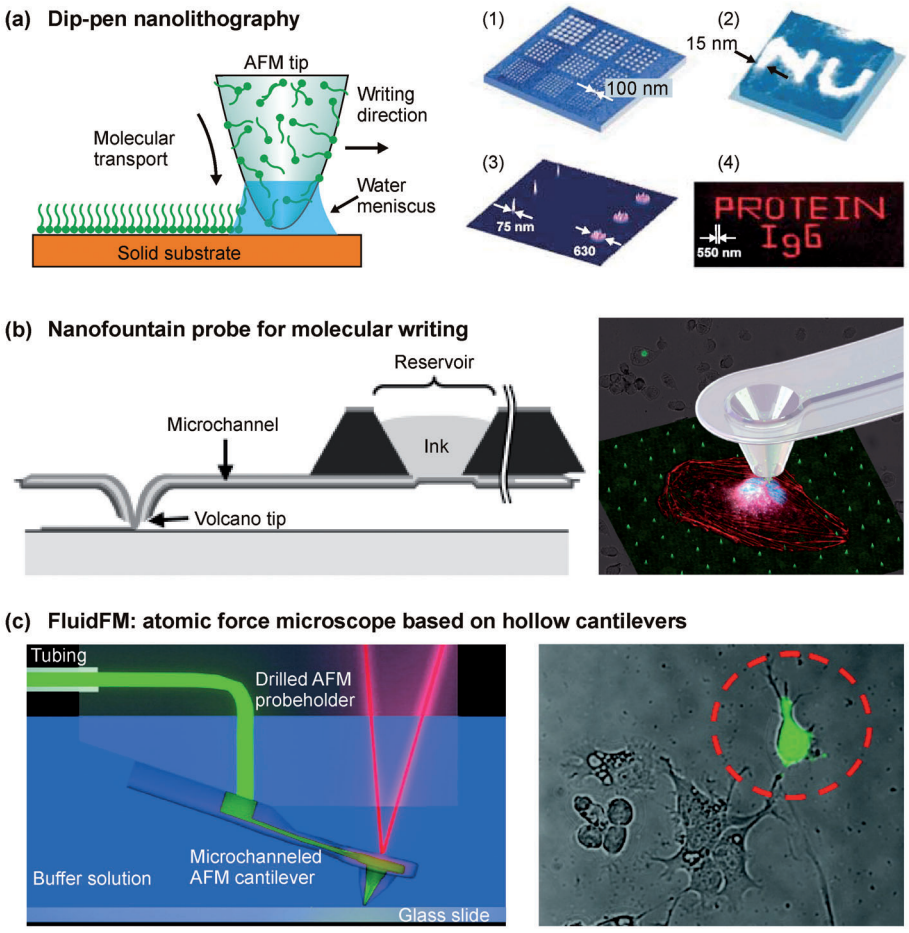

Figure 3. Scanning probe microscopy for local processing and delivery of chemicals to biological interfaces. a) In DPN, ink migrates from the surface of an AFM tip to a surface in the area of contact. Using DPN, 1) nanoscale dot arrays, 2) letters written on a gold surface, 3) 25 nm and 13 nm gold nanoparticles hybridized to surface DNA templates, and 4) patterns of fluorescently labeled immunoglobulin G were written. b) With a nanofountain probe, ink fed from a reservoir forms a liquid—air interface at the tip and molecules are transferred by diffusion from the interface to a substrate. c) A cantilever fixed to a drilled AFM probe holder delivered a fluorescent stain to a single living neuron. Images reproduced with permission and their source: (a) Left image reproduced from Ref. [29], images (1) to (4) from Ref. [30], copyright 2009 American Chemical Society. (b) Reproduced from Ref. [31]. (c) Reproduced from Ref. [32], copyright 2009 American Chemical Society.

contact mode, but precise control of the distance between the cantilever and the surface is critical. This imposes limitations on the roughness and elasticity of the surfaces that can be probed as well as on the lateral displacement speed of the cantilever.

An attractive way to deliver chemicals in DPN is to use microfluidic elements to direct the liquid to the probe. The Espinosa group[31,33] developed an approach for continuous feeding of inks to the tip of the probe. They developed an AFM tip in which an aperture was implemented at the apex of a hollow pyramidal reservoir (Figure 3b).[31] They called this device nanofountain pen and it comprises a volcano tip, microchannels, and an on-chip reservoir. In this design, the ink fed into the reservoir is driven by capillary action through the microchannels to form a liquid–air interface around the tip. Patterns with lines as small as 40 nm were written.

Similar to the implementation of a "reservoir" for liquids in the vicinity of the cantilever, Zambelli and co-workers[32] integrated microfluidic elements onto an AFM setup and called their technology FluidFM (Figure 3c). This device comprised a hollow cantilever with microfluidic elements for use in air and liquid. The array of cantilevers was connected to a delivery system by a modified AFM probe holder, enabling force-controlled dispersion of a solution containing selected molecules into individual cells within a physiological environment. The cantilever geometry and the AFM force feedback allowed local delivery of molecules to cells either by gentle contact with their membrane or by perforation of the membrane. The FluidFM technology also made it possible to select microscopic objects, such as living mammalian cells, yeast, or bacteria.

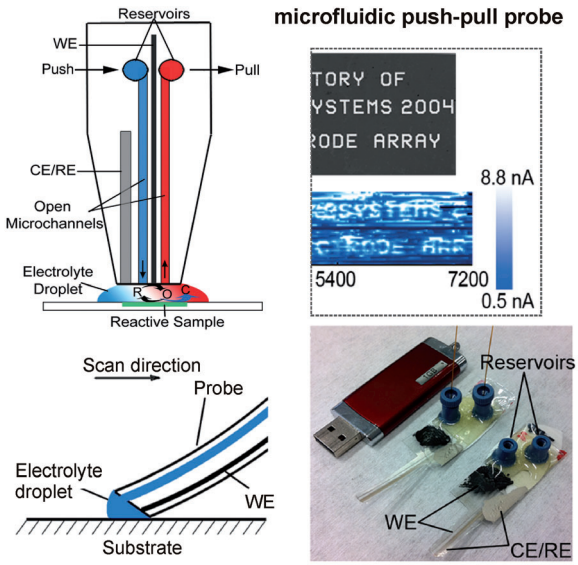

Figure 4. SECM techniques for local electrochemistry in liquid environments. Top left: Push—pull probe with multiple channels for injecting and aspirating electrolytes; top right: an image of a text pattern in gold bottom and the corresponding SECM image; bottom left: a sketch of an exposed probe tip; bottom right: the photograph shows two push—pull probes. Reproduced from Ref. [35], copyright 2011 American Chemical Society.

3.2. Microelectrochemistry of (Bio)Chemicals

Miniaturization and precision positioning of needle-type electrodes are rapidly enabling new means of visualization and control of chemical and biochemical reactions on surfaces. There are several excellent Reviews related to this topic, for example, the one by Schulte and Schuhmann[34] written in the context of single-cell analysis. Combining microfluidics with microelectrodes operated in scanning electrochemical microscopy mode or with nanopipettes for scanning ion conductivity microscopy enables new applications, and these hybrid approaches are reviewed below.

3.2.1. Scanning Electrochemical Microscopy (SECM)

The push–pull probe, developed by Momotenko et al.,[35] maps (electro)chemical information of surfaces with high spatial resolution. The device integrates a working electrode, a counter/reference electrode, and a microfluidic system (Figure 4). Microfluidic channels continuously replenished liquid between the probe tip and the interface hence electrochemical mapping of initially dry samples could be performed. In addition, because no immersion of the sample in electrolyte was necessary, the investigation of vertical or tilted substrates became possible. The push–pull probe was used in a "contact" mode, and therefore eliminated the time-consuming procedure of sample leveling prior to SECM imaging. This approach allowed the investigation of corrugated or curved samples. SECM images of various gold-on-glass samples provided the proof of concept for local surface activity characterization with high resolution. We believe that this probe can be coupled to other analytical techniques, such as mass spectrometry, liquid or gas chromatography, and capillary electrophoresis.

Cantilevers are suited for depositing biomolecules locally on surfaces. Bergaud and co-workers[36] leveraged this concept and developed techniques to control the assembly of oligonucleotides on a surface. They adapted the polypyrrole technology to a cantilever-based deposition system for patterning surfaces for DNA immobilization. The array of cantilevers they designed consisted of ten cantilevers for deposition of molecules and two external force-sensing cantilevers for positioning the array (Figure 5). Each cantilever comprised a microfluidic channel and a reservoir. A gold electrode was patterned within the channel to enable electrochemical reactions to take place at the tip. Electrospotting was carried out by simultaneously filling the depositing cantilevers with electrolyte prior to positioning each of them at a precise location on the surface of a gold-coated substrate. Then an electrical pulse was applied between the anodically polarized gold substrate and the cantilever for 25 to 500 ms before releasing the cantilevers. The procedure to electropolymerize oligonucleotides is shown in Figure 5. The use of a cantilever array allowed electrochemical reactions in picoliter droplets to prepare biochips.

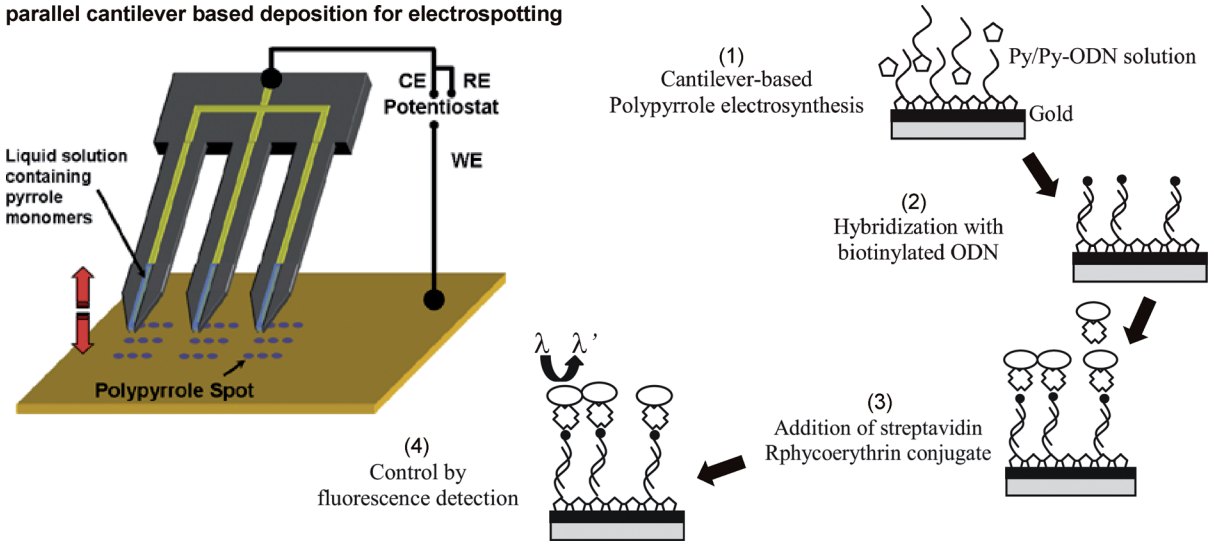

Figure 5. Cantilever array and 1)–4) the process for local electrodeposition of oligonucleotides. ODN = oligodeoxynucleotides. Reproduced from Ref. [36], copyright 2011 American Chemical Society.

3.2.2. *Nanopipettes for Scanning Ion Conductivity Microscopy (SICM)*

Nanopipettes were developed for the direct patterning of surfaces with biological material, and have since been used for numerous applications in biology. Ying[37] has published a Review on the use of nanopipettes in bionanotechnology. An early demonstration of nanopipettes by Bruckbauer et al.[38] was based on a variant of SPM called SICM, which was developed for scanning soft, nonconductive materials by using an electrolyte-filled nanopipette as probe. Key to this approach was the robust SICM distance control. The ionic current flowing between an electrode inside the nanopipette probe and an electrode in the bath was used to control the pipette-sample distance. Pipettes with inner diameters of 100–150 nm were positioned at a distance of about 100 nm above a glass surface. Biotinylated and fluorophore-labeled DNA or protein G were delivered to the surface and immobilized by biotin-streptavidin binding or electrostatic interaction. The electric field in the tip region is non-uniform along the axis of the pipette because of the conical shape of the pipette. The number of molecules exiting the tip depends on the combination of electro-osmotic flow, electrophoresis, and dielectrophoresis.

Physical investigations of cells and the delivery of selected molecules on specific regions on the cell membranes require sensitive and specialized techniques. Bruckbauer et al.[39] developed a tool for delivering fluorophore-labeled molecules in a controlled manner and, in addition, succeeded in performing single-molecule tracking. The sign and magnitude of the applied voltage controlled the number of molecules delivered. A nanopipette delivered individual fluorescent probes to preselected sites on the plasma membranes of living boar spermatozoa (Figure 6). The intent was to track the diffusion of membrane glycoproteins in different macrodomains. This technique enabled important investigations in membrane biology to study the heterogeneous distribution of component lipids and proteins. This high-resolution scanning and delivery technique also enabled the interrogation of the dynamics of submicron structures, such as lipid rafts or transient confinement zones. This was done by tracking and analyzing the diffusion of single molecules in real time, whereby the molecules could be located with a precision of 50 nm. In contrast to the use of flurophores, which photobleached in short time, the nanopipette-based approach allowed multiple experiments to be performed on the same cell to probe different macro-domains

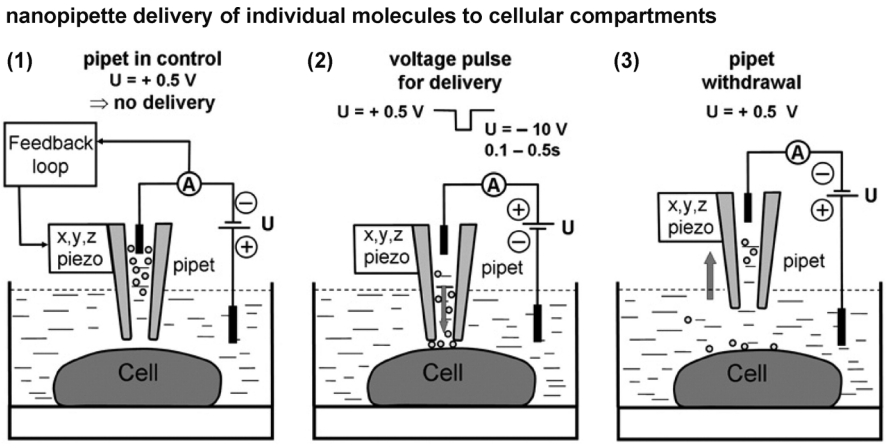

Figure 6. Working of the nanopipette. The pipette is placed at a distance of 100 nm from the cell membrane at positive potential, and then a negative voltage pulse is applied to deliver molecules to the cell membrane. Reproduced from Ref. [39], copyright 2007 Elsevier.

and sub-regions in the plasma membrane, and the investigation of the existence of diffusion barriers without being limited by observation times.

Mechanobiology is a topic of increasing importance, but the techniques capable of probing the mechanical properties of membranes without damaging them are inadequate.[40] Shevchuk et al.[41] developed a method for imaging contracting cardiac cells using nanopipettes (Figure 7a). A distance-modulated method for SICM provided a sophisticated distance-control mechanism, enabling complicated physiological experiments to be made that required alteration of the ionic strength of the liquid media. When the cardiac myocyte contracted, the feedback control moved the sample stage to maintain a constant distance between the nanopipette and the cell surface. The technique measured rapid changes in cell height from 10 nm to several micrometers with millisecond time resolution. Nanopipettes were combined with laser confocal microscopy for the simultaneous measurement of the nanoscale motion of cardiac myocytes and the local calcium concentrations under the cell membrane. Interestingly, despite large cellular movements, a simultaneous tracking of the changes in cell height and measurements of the intracellular Ca^{2+} near the cell surface were possible with the nanopipette approach while retaining cell functionality.

Imaging living cells with nanoscale resolution provides great insight into the biomolecular mechanisms of cells. However, SICM is restricted to imaging relatively flat surfaces, whereas cells do not have a flat surface. When the nanopipette encountered a vertically protruding structure during scanning, it collided with the structure. Novak et al.[42] overcame this problem by using SICM in a hopping mode. This allowed non-contact imaging of complex three-dimensional surfaces of living cells with resolutions better than 20 nm (Figure 7b). Hopping probe ion conductance microscopy (HPICM) was demonstrated by imaging nanoscale phenomena on the surface of living cells under physiological conditions. In conventional SICM, a nanopipette is mounted on a three-dimensional piezoelectric translation stage and an automatic feedback control moved the pipette up or down to keep the pipette current constant while the sample was

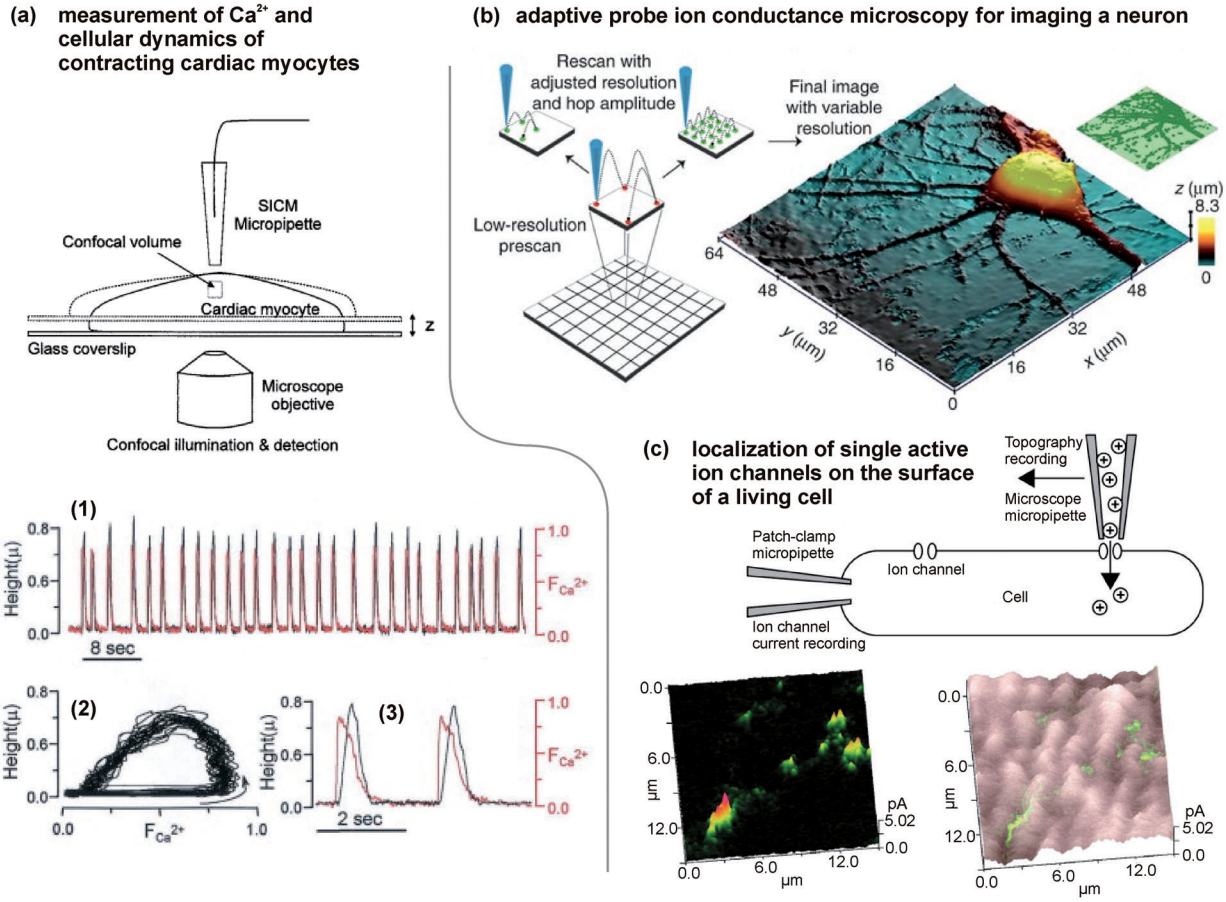

Figure 7. Nanopipettes for nanometer-scale interactions with biological interfaces. a) Simultaneous SICM and laser confocal microscopy on a contracting cardiac myocyte. Plots: 1) simultaneous measurement of the surface of a cultured rat cardiac myocyte and the local Ca^{2+} concentration below the cell surface; 2) cell height versus relative calcium concentration for all contractions in (1); 3) close-up of two of the contractions in (1). b) Principle of adaptive HPICM and resulting three-dimensional image of a neuron. c) Localization of single ion channels in intact cell membranes. Images are maps of K^+ current (left) and of surface topography superimposed on the K^+ current map (right). Images reproduced with permission and their source (a) Ref. [41], copyright 2001 Elsevier. (b) Ref. [42], image supplied by the authors. (c) Ref. [43], copyright 2000 Macmillan Publishers.

scanned laterally. In contrast to conventional raster scanning, HPICM had the advantage that the order of imaging pixels did not have to be predetermined. The entire image was divided into equal-sized squares and, prior to imaging each square, the overall roughness of the sample was estimated by measuring the difference in height at the corners of the square. If the sample in a square was rough, the topography of that square was then measured at high resolution. If the sample was relatively flat, the squares were imaged at lower resolution. This adaptive technique was demonstrated by imaging of hippocampal pyramidal neurons in 15 min (Figure 7b). It is very likely that this technique will increasingly be applied to biophysical studies of cells.

Biophysical studies of cell surfaces require techniques to map single active ion channels in intact cell membranes. This type of mapping is important because the spatial distribution of ion channels in the cell membrane plays a key role in providing precise and localized information on cellular functions. Using nanopipettes, Korchev et al.[43] mapped the distribution of ATP-regulated K^+ channels (K_{ATP} channels) in cardiac myocytes (Figure 7c). The channels were organized in small groups and anchored in the Z-grooves of the sarcolemma. The distinct pattern of the channel distribution has important functional implications. This scanning method showed that the K_{ATP} channels hold their position on the cell surface for a relatively long time, and has the potential to be used for a range of precise biophysical investigations on cells.

3.3. Transport Based on Multiphase Systems

Multiphase systems often are involved in compartmentalization in nature and essential for biochemical processes.[44] In microfluidic systems, compartmentalization has been achieved using immiscible liquids. Closed-channel microfluidics exploit immiscible systems. Particularly appealing about this strategy is that it requires only inexpensive materials and off-the-shelf equipment, and that it is easily accessible to researchers without specialist microfabrication expertise. A Review by Baret[45] provides insight into multiphase systems using microfluidic systems; the comprehensive Review on aqueous two-phase systems by Hardt and Hahn[46] complements it. In this Section, we Review the demonstrations in open microfluidic systems that leverage multiphase and immiscible systems.

Key aspects of using multiphase systems are the diffusive mass transfer and sample partitioning between two or more phases. Such systems can qualitatively be understood by considering the Gibbs free energy (ΔG_{mix}) of mixing governed by $\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix}$, where ΔH_{mix} is the enthalpy difference and ΔS_{mix} the entropy difference between the mixed and the phase-separated state. The timescale of the partitioning depends on the diffusion coefficient and may be on the order of several hours. Control of surface energy is important in partitioning within multiphase systems.

3.3.1. Polymeric Aqueous Two-Phase System (ATPS)

Takayama and colleagues contributed extensively to the implementation of aqueous two-phase systems in microfluidics. They explored polymers such as polyethylene glycol (PEG) and dextran dissolved in aqueous solutions because 1) these polymers form stable ATPSs over a wide range of temperatures, thereby increasing the convenience and stability of experiments; 2) the high molecular weight of PEG and dextran form ATPSs at low polymer concentrations ensures that the bulks of both phases remain highly aqueous and nontoxic to cells, and 3) this system works robustly in commonly used cell culture media. In Figure 8a, the interfacial free energies between the two aqueous phases, the cell layer, and the PEG phase, and between the cell layer and dextran phase are represented by γ_{12}, γ_{C1}, and γ_{C2}, respectively, and the contact angle between the three phases is represented by θ.

Exposing selected cells in culture to reagents is important, for example, in single-cell transfection, in toxicity studies, and for creating living cell microarrays. Tavana et al.[47] loaded a pipette tip with the dextran phase and lowered it into the PEG phase in proximity to a cell monolayer. Moving the pipette tip horizontally resulted in the formation of a continuous pattern of the dispensing dextran phase on the cells. This was demonstrated by patterning "UMICH" on a monolayer of HEK293H cells (Figure 8b). The patterns were found to be stable over long incubation times. Critical for the stability of patterns is the low interfacial energy between the two immiscible phases and the roughness of the cell culture and the associated cell surface-dextran phase. The patterning technology was demonstrated with multiplexed cell-based studies of gene expression and gene silencing.

The generation of heterocellular niches by spatial patterning of one type of cells onto another cell type will likely be useful for a range of biological studies, including stem-cell research and regenerative medicine. To this end, Tavana et al.[48] used ATPS for non-contact printing cellular patterns on living cells in physiological environments. They engineered cellular niches to support neuronal differentiation of mouse embryonic stem cells (mESC) and showed that the density of printed mESC was an important factor for guiding mESC differentiation to neurons (Figure 8c).

Platforms to study the role of cell–cell contact on various cellular phenotypes may strongly support numerous aspects of stem-cell research. In the work by Tavana et al.,[49] droplets of cell suspension in the denser aqueous phase were printed onto a substrate residing in an aqueous immersion phase. Because of their affinity for the denser phase, cells remained localized within the drops and adhered to regions of the substrate below the drops. Printing two different supporting cell types onto a gel surface created duplex heterocellular stem-cell niches, which were overlaid with mESCs. To achieve multiple cell microarrays, the two cell types were mixed separately with the dextran phase, and the suspensions was transferred into a 1536-well plate (Figure 8d). Slot pins mounted to a fixture were dipped into the wells to load the cell-containing dextran phase. The pins were then withdrawn from the source plate and dipped into a culture dish containing the PEG phase. The aqueous two-phase cell

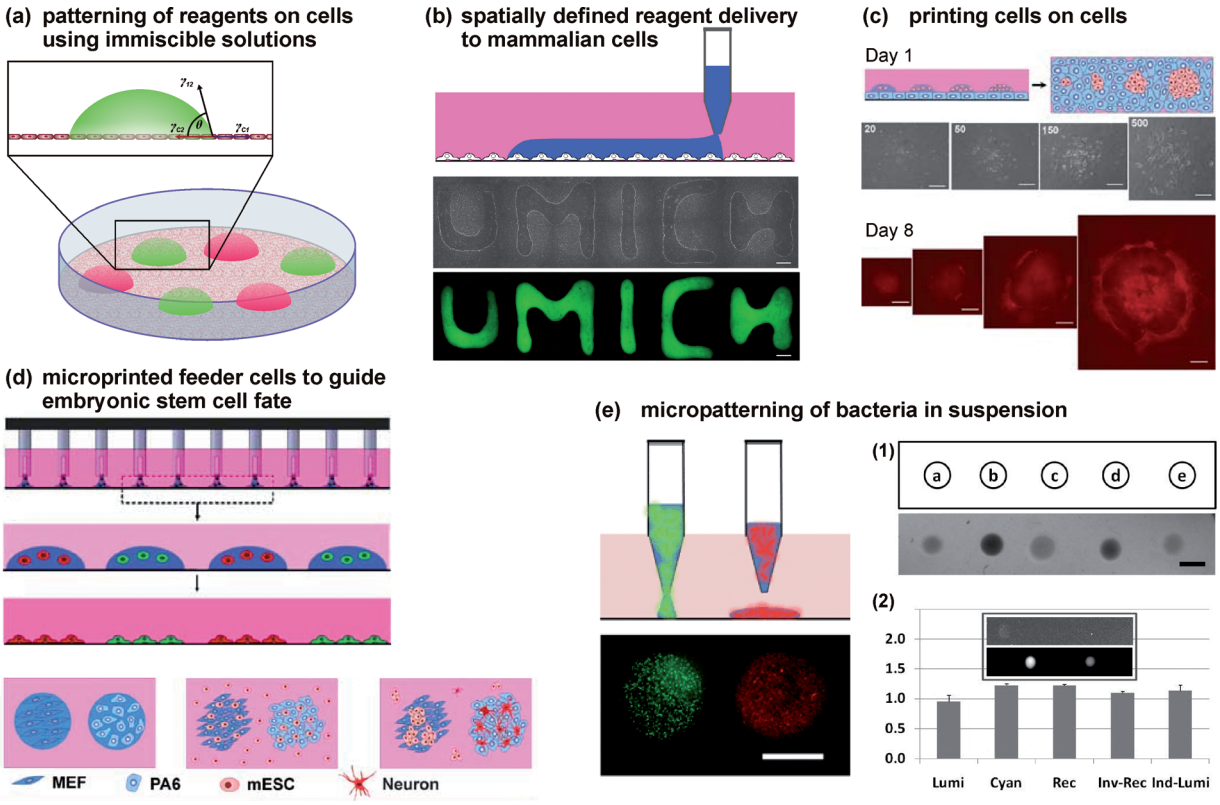

Figure 8. Aqueous multiphase systems for patterning reagents or cells atop cells. a) Dextran droplets containing reagents, either green or red dye in a PEG solution, were patterned on living cells. b) Patterning of an aqueous dextran phase (blue) on a cell monolayer covered with the PEG phase (pink). Bright-field and fluorescent images of a patterned dextran phase on HEK293H cells spelling out "UMICH". c) Colony-size dependent neuronal differentiation of mESC on PA6 supporting cells. Schematic and bright field/fluorescent images of mESC printed on a PA6 monolayer at different densities. d) Multiplexed aqueous two-phase cell printing. e) Different bacteria (cyan or red fluorescent) suspended in dextran positioned inside a dish filled with a PEG-rich phase to pattern bacterial suspensions. The optical micrograph (1) of the bacterial suspensions and the graph (2) show the change in luminescence of the bacterial suspension without chemical stimulation. See text for details. Images reproduced with permission: (a) Ref. [9]; (b) Ref. [47], image supplied by the authors; (c) Ref. [48]; (d) Ref. [49]; (e) from Ref. [53], image supplied by the authors.

culture media nourished cells during incubation. After the cells attached, the ATPS were washed and replaced with regular culture media. This process resulted in uniformly sized islands of two different cell types with defined spacing. How the supporting cells were printed spatially determined the fate of the overlaid mESCs. The mESCs colonies placed on differentiation-inducing feeder cells exhibited enhanced neuronal differentiation and resulted in dense neuron networks.

Cell-to-cell communication in bacteria, called quorum sensing, allows bacteria to monitor their environment.[50,51] Studying quorum sensing in the "open" space is representative of the true environment encountered by bacteria. Physically segregated, but chemically connected patterns of different bacterial populations are useful for studying the functions of bacterial and other cell populations.[52] Interrogation of such quorum sensing is challenging because bacteria in suspension typically disperse due to diffusion, convection, and the motility of the bacteria themselves, which precludes spatial confinement. Yaguchi et al.[53] developed a stable spatial patterning of sub-microliter droplets of bacterial suspensions using ATPS. Different types of bacterial populations were positioned and maintained adjacent to each other without dispersion (Figure 8e). ATPS was used to localize high concentrations of *E. coli*. When a chemical stimulus was applied, the droplet array produced a pattern of bacterial "illumination" that reflected the type of chemical to which the array was exposed. Microcolonies were created with different bacterial densities. Each microcolony essentially became a biosensor and, depending on the type of bacteria suspended, different chemicals could be sensed. As shown in Figure 8e, the droplet were expected to a) constitutively express bioluminescence, b) constitutively express cyan fluorescence protein, c) increase the expression of green fluorescent protein in response to acyl homoserine lactone, d) decrease the expression of green fluorescent protein in response to acyl homoserine lactone, and e) increase the bioluminescence in response to mutagens. Figure 8e shows the micrographs and plots of the response of the bacterial biosensor array.

3.3.2. Other Partitioning Approaches

The demonstrations in the preceding Section highlight the numerous advantages of multiphase systems for compartmentalization in the "open" space. Ismagilov and co-workers[54] developed a device they called chemistrode and which

combined multiphase systems with scanning probes. This microfluidic device used immiscible liquids to generate plugs to spatially separate liquids and enabled stimulation, recording, and analysis of molecular signals with high spatial and temporal resolution. The chemistrode therefore brought liquids transiently into contact with a chemical or biological substrate, and molecular signals were exchanged. Such an analytical tool could be useful to understand systems that rely on molecular signals, for example, chemical communication between cells. The chemistrode does this by chemical stimulation and recording of short pulses of 50 ms. Molecular signals are delivered by and captured in aqueous plugs surrounded by a fluorocarbon carrier fluid. The compartmentalization of these molecular signals eliminates dispersion and loss of sample by surface adsorption. Recorded molecular signals were injected with additional reagents and analyzed off-line. When recombined, such analyses provided a "time stamp" on the chemical record of a system's response to stimulation. As shown in Figure 9, insulin secretion from a single murine islet of Langerhans cells was measured using the chemistrode. The operation of the chemistrode consists of the following steps: 1) preparation of an array of aqueous plugs containing an arbitrary sequence of stimuli, 2) delivery of the array of stimulus plugs to a hydrophilic substrate, 3) coalescence of the stimulus plugs with the wetting layer above the hydrophilic substrate while the fluorocarbon carrier fluid remains in contact with the hydrophobic wall of the chemistrode, 4) rapid exchange of diffusible signals between the plugs and the wetting layer, 5) delivery of response plugs to a splitting junction to form identical daughter arrays, 6) injection of each daughter array with reagents required for further analysis, 7) analysis of each daughter array by a different technique, 8) and the recombination of data from the analysis of daughter arrays to provide a time-resolved record of molecular stimulation and response dynamics. This approach is therefore useful to measure molecular signals from multiple locations on a biological interface with high spatial resolution.

3.4. Hydrodynamic Focusing of Liquids

Hydrodynamic focusing of liquids is well established in "closed" channel devices and routinely used in flow cytometers and Coulter counters for counting and determining the size of cells and particles. In this Section, we review microfluidic approaches that leverage hydrodynamic focusing in the "open" space (Figure 10 and 11).

3.4.1. Microfluidic Probe (MFP)

The first to report on the microfluidic probe (MFP) was the IBM group in Zurich.[55] The MFP combines hydrodynamic focusing with the concept of scanning probes for processing surfaces. The liquid boundaries formed by hydrodynamic forces underneath a MFP head confine a flow of "processing" solution. This situation is realized by setting the aspiration flow rate (Q_a) of the processing solution to be greater than the injection flow rate (Q_i; Figure 10a). The base configuration of the head has two micrometer-sized channels that injected/aspirated liquid. The surrounding liquid within which the MFP head is immersed, called immersion liquid, encapsulates the processing liquid. Therefore hydrodynamic focusing replaces the solid walls of closed microchannels, enabling the transition from closed microfluidics to microfluidics in the "open" space. In addition, because the MFP is mobile it can process large areas by scanning across them in an interactive manner. The liquid flows in this system are characterized by low Reynolds number (a measure of the ratio of inertial to viscous forces for a given flow condition) and high Péclet number (a measure of the convective versus diffusive transport, which helps to evaluate whether diffusion can broaden the hydrodynamically confined processing solution). Therefore, diffusion of the input liquid into the surrounding liquid is minimal, which enables local processing of biointerfaces in

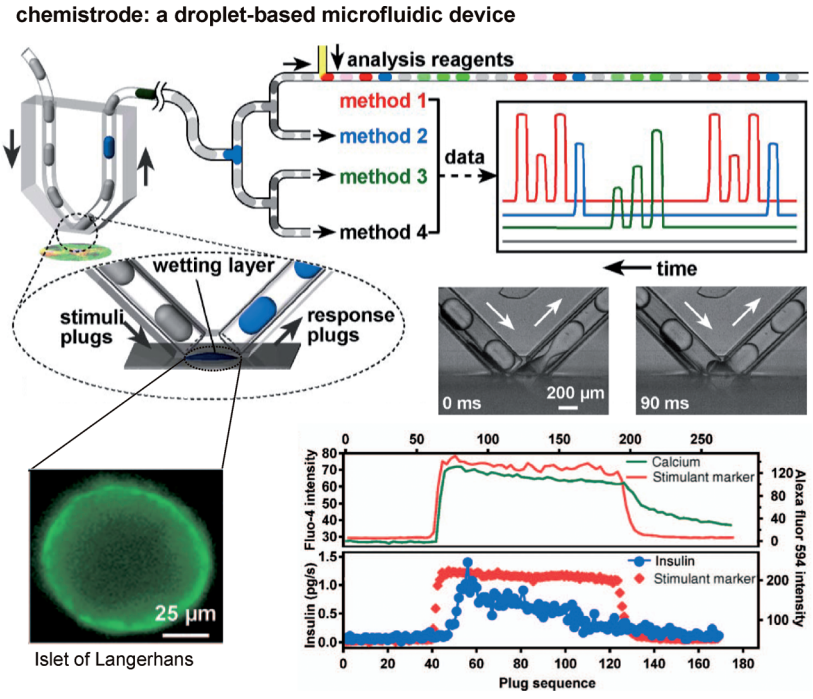

Figure 9. Schematic of a chemistrode in contact with a hydrophilic substrate showing how samples can be analyzed by several different methods. A fluorescent image of an islet showing an increase in fluorescence, corresponding to the rise in intracellular $[Ca^{2+}]_i$ (*i* indicates intracellular) in the islet upon simulation with the chemistrode. Right: time-lapse bright-field images of an incoming stimulus plug merging with the wetting layer. The plots indicate the $[Ca^{2+}]_i$ response and insulin secretion of a stimulated cell islet. Traces measured by fluorescence microscopy during stimulation and recording showing fluorescence intensity of green dye as an indicator of $[Ca^{2+}]_i$ and the intensity of red dye as a marker of the stimulant solution (top right). Traces with results of off-line analysis of plugs collected during recording (bottom). Reproduced from Ref. [54].

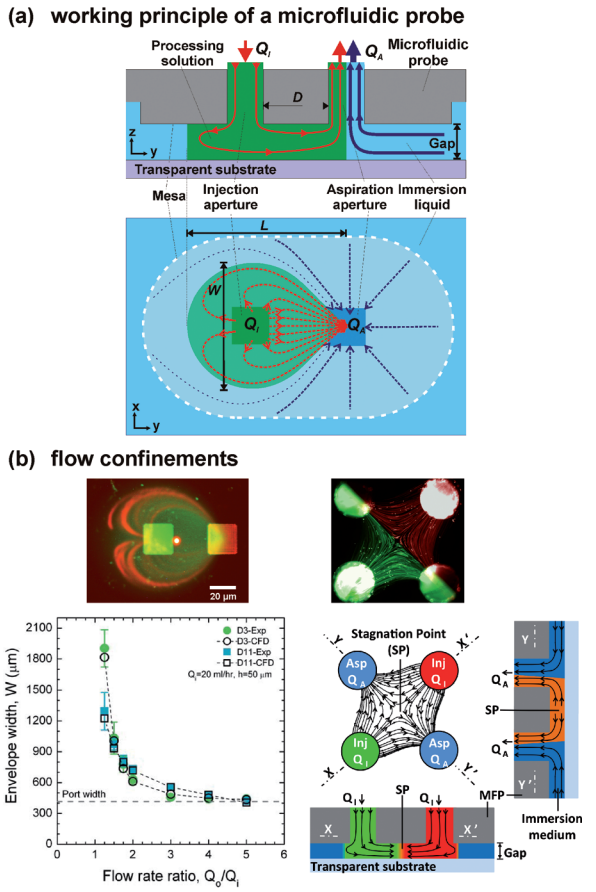

Figure 10. Hydrodynamic flow confinement: principle and microfluidic probe heads. a) Principle of the MFP. b) Micrograph of a flow confinement and plot showing the dependence of the confined liquid on the injection/aspiration flow rates. MFPs with multiple apertures were used to generate complex shapes of flow confinements and "floating" chemical gradients (right). c) Various MFP heads. (a) and (b) top left reproduced from Ref. [55], copyright 2005 Macmillan Publishers; (b) bottom left from Ref. [62], copyright 2011 Royal Society of Chemistry; (b) right top and bottom, and (c) bottom right from Ref. [61], image supplied by the authors; (c) top from Ref. [56], image supplied by the authors; (c) bottom left from Ref. [58], copyright 2011 American Chemical Society.

the "open space." The size and shape of the confined liquid also depended on parameters, such as viscosity, density, interfacial tension between liquids, in addition to the aperture geometry and the gap between the probe and the biological interface (Figure 10b). The MFP allows the aspirated solution to be used for further analysis, similar to the techniques employed in perfusion or microdialysis. This tool was used to create chemical gradients on surfaces, protein microarrays, localized staining of cells, and the contact-free detachment of single cells (Figure 11a). The MFP heads were fabricated in PDMS to be able to rapidly redesign the heads for different applications. To improve reliability and yield in fabrication, Lovchik et al.[56] developed multilayered MFP heads that used

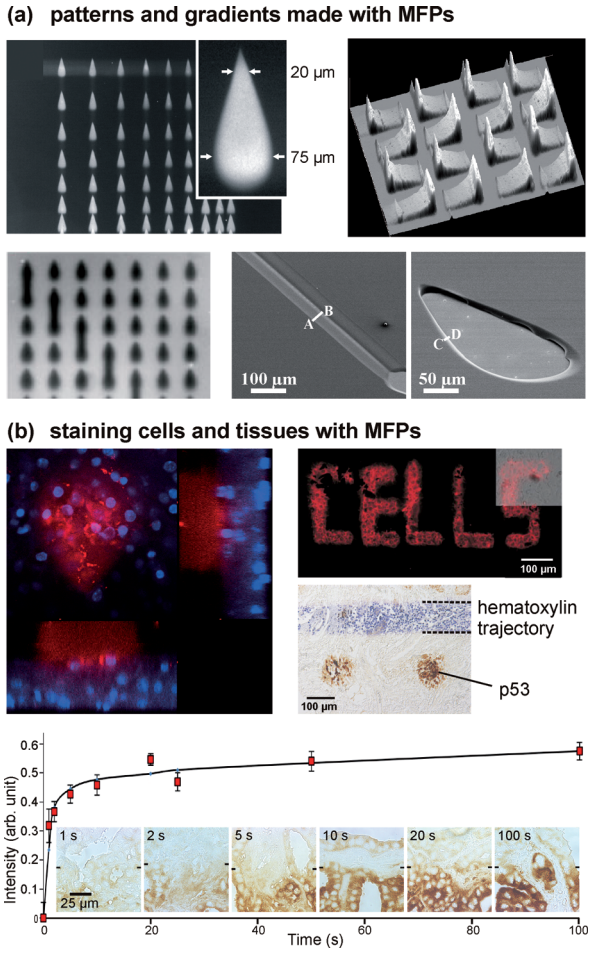

Figure 11. Localized (bio)chemistry using microfluidic probes. a) MFP-based local deposition of molecules (top left), complex gradients (top right), removal of proteins (bottom left) and structuring of photoresist (bottom right). b) MFPs were used to perform local perfusion of brain cells (top left), staining confluent cells with dyes (top right) and local immunohistochemistry on cancerous tissue sections (middle right). Multiple regions of a normal human thyroid tissue section were incubated with α-TGB for various residence times and the resulting staining intensities for various residence times are shown in the plot (bottom). The marks in the micrographs (insets within the plot) indicate the boundary between stained (lower half) and unstained regions (upper half). (a) top right and bottom left and (b) top right reproduced from Ref. [55], copyright 2005 Macmillan Publishers; (a) top left and bottom right from Ref. [56], image supplied by the authors; (b) top left from Ref. [60], image supplied by the authors; (b) center right and bottom from Ref. [59], image supplied by the authors.

standard microfabrication processes, ports and fittings. These multilayer heads were demonstrated by depositing antibodies on surfaces and by directly developing a layer of photoresist. Perrault et al.[57] subsequently published a description of an MFP platform that largely focused on the instrumentation perspective.

With the goal of developing the MFP for broad application to biological interfaces, the IBM group developed a vertically oriented MFP, the vMFP.[58] The vMFP overcomes challenges that limited the practical implementation of the original MFP technology.[55] The device consists of glass and silicon with the microfluidic features fabricated in-plane in the silicon layer. The vMFP head was oriented vertically, with the apex parallel to the surface and typical gaps of 1–30 μm. Several design and implementation strategies to achieve high yield in fabrication of the vMFP heads were reported. In addition, they also integrated functional elements, such as particulate filters, redundant aperture architectures, inclined flow-paths, and multiple channels to enable symmetric flow confinements into the vMFP heads. A method to calibrate the distance between the apex of the vMFP and the biological interface was established. This approach involved visually monitoring the confinement of a solution containing fluorescently labeled antibodies on a glass surface while changing the gap distance between the probe head and the surface. The versatility of the vMFP was shown by patterning proteins on surfaces, inactivation of cells using sodium hypochlorite, and staining living NIH fibroblasts.[58]

More recently, the IBM group applied the vMFP for multiplexed and precise staining of tissue sections.[59] They called their technique micro-immunohistochemistry (μIHC). This staining approach circumvents typical challenges encountered by pathologists, such as limited tissue samples and the need for qualitative staining results. The incubation of primary antibodies was performed using the vMFP, which is an important step in immunohistochemistry. In μIHC, nanoliters of primary antibody solutions were confined over micrometer-sized areas of tissue sections using the vMFP. This method was conservative of tissue and reagents, alleviated antibody cross-reactivity issues, and allowed a wide range of staining conditions to be applied on a single tissue section. The vMFP head was compatible with (bio)chemical systems used for staining and resistant to a range of chemicals and could be used indefinitely unless damaged physically. Its shape and its small apex enabled easy observations of tissue sections from both above and underneath using an inverted microscope. As expected, a correlation between the incubation time of the primary antibodies and the signal intensity was observed (Figure 11b). This correlation revealed that specific interactions between antigen/antibodies from various tissues could be adjusted by varying the residence time of the MFP head on the tissue section. Incubation of anti-thyroglobulin (α-TGB) with thyroid tissue was tested to achieve visible staining and the necessary incubation time was found to be on the order of 20 s for the conditions used in the experiment. This incubation duration is much shorter than the 30 min recommended for conventional staining, a reduction that was attributed to the enhanced convective flows generated by the vMFP. Multiplexed μIHC was also performed on cancerous breast tissue sections and on selected cores of a tissue microarray.

Microfluidic approaches are well suited for culturing and studying single cells; however, the culturing of organized tissue, such as brain slices, continues to be difficult in microfluidic devices. Queval et al.[60] used the MFP to perform local perfusion of brain tissue. This perfusion allows pieces of brain tissue to be maintained in vitro for extended durations without changes in the cellular composition of the brain tissue. This allowed many biological processes that occur in the course of days to weeks in the brain tissue to be studied, while also enabling high-resolution imaging using confocal microscopy. The setup for this comprised a perfusion chamber for the culture of organotypic slices and a transparent MFP for the microperfusion of the brain tissue. The MFP used was made from PDMS and had six apertures to perform perfusion of a small number of cells in a brain (Figure 11b).

Extending the single aperture pair in the MFP to multiple pairs not only parallelized the number of local chemical reactions, but also enabled the creation of stable soluble gradients in solutions. Qasaimeh et al.[61] developed such a two-dimensional microfluidic quadrupole (MQ) consisting of four apertures, and generated local floating chemical gradients. The MQ was formed by simultaneously injecting and aspirating fluids from two pairs of opposing apertures in a narrow gap formed between the microfluidic probe and a substrate (Figure 10c). Because of the multiple flow confinements, a stagnation point formed at the center of the microfluidic quadrupole, as expected, and its position was adjusted hydrodynamically . The floating gradients enabled rapid spatiotemporal tuning of the gradient either hydrodynamically by adjusting the flow rates or physically by moving the MFP. MQs could easily be applied on different biological interfaces.

Although several applications of the MFP have been demonstrated, the design and operation of the MFPs were largely guided by empirical optimization. While Qasaimeh et al.[61] reported a theoretical framework to generate microfluidic quadrupoles using the MFP, Christ and Turner[62] provided insights into the underlying fluid mechanics. They undertook a comprehensive study to establish the relationships between the device geometry, inlet and outlet flow rates, and the fluid physics. They investigated the effect of multiple flows and aperture design parameters on the size and shape of the flow envelope and the pressure drop between the apertures of several two-aperture devices. Trends were established based on 3D computational fluid dynamic predictions and experimental measurements for several geometries of devices with a range of gap heights, flow rates, and flow rate ratios. Their analytical and simulation results provided an elegant framework for understanding the fluid mechanics of MFPs and will likely aid in the design of the future MFPs.

3.4.2. *Dual/Multi Capillary Probes*

Hydrodynamic focusing of liquids in the "open" space has also been performed using dual or multiple capillaries.

Several interesting demonstrations used such capillaries to hydrodynamically confine liquids and are described in this Section.

Collecting bioanalytes from selected cells in culture is generally challenging. Shiku et al.[63] implemented a dual capillary probe for mRNA analysis of selected adherent cells. This probe comprised theta-shaped glass capillaries. A cell lysis buffer solution was introduced from an injection aperture without affecting cells in the vicinity, and the cell lysate was collected through the aspiration aperture for analysis (Figure 12a). Human breast cancer cells NCF-7 and malignant human mammary epithelial cells HMT-3522 T4-2 were used, and the efficiency in collection of the mRNA was evaluated with real-time polymerase chain reaction (PCR).

Monitoring ion-channel activity of single cells is critical in electrophysiological investigations to understand the functioning of a cell. Ainla et al.[64] developed a microfluidic-pipette-based device made from PDMS having a tip in which circulating liquids generated a self-confining volume in front of the outlet channels (Figure 12b). More recently, this group published a sophisticated version of this device, which handled multiple liquids streams and allowed a rapid switching of solutions.[65] The device used flow recirculation of liquids to eliminate cross-contamination in the "open" space and enabled spatial control of the concentration of chemicals applied to selected objects on the surface. The tip dimensions were defined by the recirculating fluid and were less than 10 μm to address single cells. Using an uptake assay, in situ dose-response curves from adherent Chinese hamster ovary cells expressing proton-activated hTRPV1 receptors were shown. With confined superfusion and cell stimulation, hTRPV1 receptors in single cells were activated, and the response measured by a patch-clamp pipette (Figure 12b). Another implementation of a dual pipette-based system was that of Feinerman and Moses,[66] which was developed for local and controlled drug infusion. Their system consisted of two concentric pipettes, in which each could be manipulated separately and pressurized independently. The inner pipette was loaded with the desirable solution and functioned as a source, whereas the outer one was a sink. This enabled a flow of the solution between the two pipettes that protruded only a small distance into the surrounding fluid. Time resolution of the infusion was found to be highly controllable, and oscillatory flows were generated, if required. This was useful for certain implementations in drug dosing. A similar PDMS-based MFP was recently used by Han and co-workers[67] to measure cell kinase activity in adherent cells.

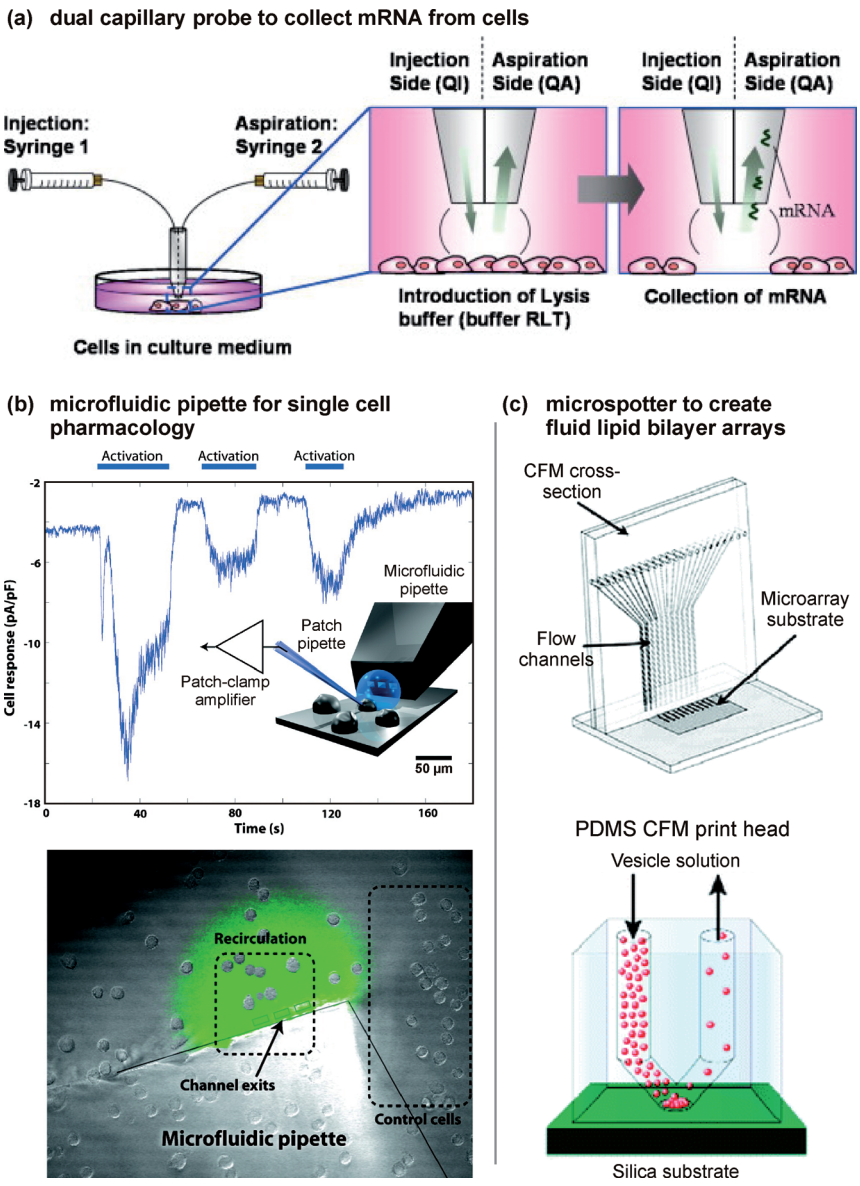

Figure 12. Dual/multi pipettes for localizing liquids. a) Theta-shaped microfluidic dual capillary probe used to lyse selected cells and retrieval of cell lysate. b) Single-cell patch–clamp implementing a fluorescence uptake assay. The plot shows the current response versus time from repeated activations of selected ion channels from Chinese hamster ovary cells. The liquid recirculation selectively exposes single cells to a chemical membrane blebbing solution (right). c) CFM apparatus and close-up of the print head in contact with a substrate used for bilayer formation. Images reproduced from (a) Ref. [63], copyright 2009 Elsevier Inc. (b) Ref. [64], copyright 2010 American Chemical Society. (c) Ref. [68], copyright 2008 American Chemical Society.

Another approach of local chemistries was shown by Smith et al.,[68] who used a 3D microfluidic system to create micropatterned lipid bilayer arrays to study protein–ligand and protein–membrane interactions in cell membranes. Micropatterned lipid bilayer arrays have been used for

biophysical investigations and sensors.[69] The device maintained characteristics found in cellular membranes, such as fluidity and biocompatibility. An array of fluid lipid membranes was patterned onto glass substrates using a continuous flow microspotter (CFM; Figure 12c). Fluorescence microscopy was used to verify the formation of a bilayer on a glass substrate. Fluorescence recovery after photobleaching experiments demonstrated the fluidity the bilayers while being individually corralled on the substrate. The reproducibility of bilayer formation was demonstrated by the linear response of membrane fluorescence versus rhodamine-functionalized lipids incorporated into vesicles prior to fusion with the surface. The flexible nature of the micropatterned lipid bilayer arrays was demonstrated with several different fluorescently labeled lipids to generate a multi-component lipid array. Related techniques, such as microcontact printing have been used to create lipid bilayers for studies of membrane biophysics, high-throughput screening of compounds, and probing living cells with synthetic membrane interactions.[70]

A variant of the MFP/dual pipette was developed by Routenberg and Reed[71] and applied to process semiconductor wafers locally. They also called their device an MFP; however, there is a fundamental difference between this device and the MFP first developed.[55] The MFP of Routenberg and Reed[71] worked in a "contact mode", it temporarily established a fluidic path when the probe was in contact with the surface. The probe is used in a fashion analogous to an electrical needle probe in which a micro-positioner serves to bring the probe into mechanical contact with the wafer to form a sealed channel. Fluids enter and exit through tubing attached to the top of the probe. Because there is no need to permanently bond the probe tip to the wafer or to rely on self-adhesion, the contact area has to be only slightly larger than the desired fluidic channel. This device was used for nanowire field-effect transistor sensor measurements.

There are several ways of fabricating MFPs and capillary probes, ranging from the use of polymers to established microtechnologies. Corgier and Juncker[72] recently developed a simple and manufacturable approach to fabricate such a device, namely, a polymeric microfabricated electrochemical nanoprobe (MEN) with nanometer-scale electrodes. The probe and the electrodes are formed by embedding nanometer-thick metal lines between layers of UV-curable adhesive polymers, and then the MENs are diced and detached. With the MENs, hydrodynamically confined shapes of liquids could be generated to interact with biological interfaces.

4. Outlook

A surprisingly large variety of problems and applications require local processing of biological interfaces, many of them having only recently been reported. It is therefore timely to review the literature on microfluidics with a focus on what can be done with local (bio)chemistries on biological interfaces. This Review captures the essence of this emerging trend and lists a number of relevant tools and techniques. There are many variants in terms of how such devices that perform microfluidics in the open space are implemented. Several scientific breakthroughs have already been made using microfluidics that operate without closed channels. Such a shift from the technical implementation to applying the technology to ever more challenging problems indicates a quick maturation of the field.

We expect significant merit in combining a range of analytical techniques on biological interfaces while performing microfluidics in the open space. This approach will likely be used in pharmaceuticals, biotechnology, and diagnostics. Some analytical methods already being applied to biological interfaces are local surface plasmon resonance to detect molecular binding events and changes in molecular conformation,[73] surface-enhanced Raman spectroscopy to sense biomolecular interactions in real-time,[74] and secondary ion mass spectrometry as well as laser-induced breakdown spectroscopy for local material and tissue analysis.

In a world where liquid handling is inherent to life sciences and medical research, microfluidic technologies to control liquid dispensing and handling over several orders of magnitude (microliter to femtoliter) and with micrometer positioning accuracy will be developed and become ubiquitous. It is only a matter of time until this happens. We are convinced that techniques capable of interacting, manipulating, and processing biological interfaces under physiological conditions will profoundly impact the fields of surface chemistry, biophysics, diagnostics, medicine, and biology.

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