Microfluidic Devices for Bioapplications

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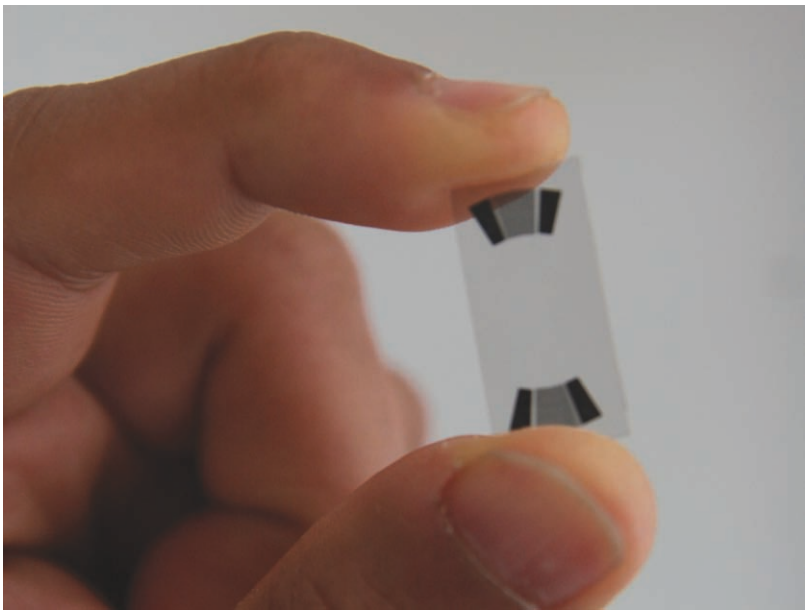

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Harnessing the ability to precisely and reproducibly actuate fluids and manipulate bioparticles such as DNA, cells, and molecules at the microscale, microfluidics is a powerful tool that is currently revolutionizing chemical and biological analysis by replicating laboratory bench-top technology on a miniature chip-scale device, thus allowing assays to be carried out at a fraction of the time and cost while affording portability and field-use capability. Emerging from a decade of research and development in microfluidic technology are a wide range of promising laboratory and consumer biotechnological applications from microscale genetic and proteomic analysis kits, cell culture and manipulation platforms, biosensors, and pathogen detection systems to point-of-care diagnostic devices, high-throughput combinatorial drug screening platforms, schemes for targeted drug delivery and advanced therapeutics, and novel biomaterials synthesis for tissue engineering. The developments associated with these technological advances along with their respective applications to date are reviewed from a broad perspective and possible future directions that could arise from the current state of the art are discussed.

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1. Introduction

Microfluidics is the science of manipulating and controlling fluids and particles at micron and submicron dimensions and the technology associated with the development of methods and devices to undertake such. Since its origins in the early 1990s, around about when microscale analytical chemistry techniques were gaining popularity and when microelectronic technology began to be recognized as a way to fabricate miniaturized chromatographic and capillary electrophoresis systems, microfluidics has grown tremendously and rapidly, sustained by the promise it offers to revolutionize conventional laboratory handling, processing, and analytical techniques. While there are many obvious possible applications for microfluidics given its potential to significantly reduce sample volumes and to carry out reaction, separation, and detection quickly and sensitively at a fraction of the typical duration and cost, the main driving force behind the rapid development of microfluidic systems has by far been its potential to be exploited for a wide range of biological applications such as high-throughput drug screening, single cell or molecule analysis and manipulation, drug delivery and advanced therapeutics, biosensing, and point-of-care diagnostics, among others. This bias in the development of microfluidic devices toward biological-related applications has arisen, in part, due to the search for a cost competitive 'high-value killer application' that can be imposed as a first-user premium so as to mitigate the risks and costs associated with the attempt to introduce any new technology into the commercial market.[1]

Moreover, not only does it seem quite logical to design systems for handling cells, molecules and other biological entities with characteristic length scales commensurate with their dimensions[2] (see, for example, **Figure 1**), it is often the case that techniques at conventional laboratory scales to carry out these procedures simply do not work or take a very long time. As an example, diffusion times, which are proportional to the characteristic system length scale squared, can be reduced by at least a million-fold simply through miniaturization. In addition, most biological systems involve microscale and nanoscale physicochemical transport processes, in particular for intercellular signaling and stimuli as well as that between the cell and the extracellular matrix, such that it is often desirable to mimic the cellular environment in in vitro assays using microfluidic structures.[3]

Microfluidics also offers a number of other advantages over conventional laboratory-scale assays. The inverse characteristic length scaling of the surface-area-to-volume ratio implies that heat and mass transfer into or out of a chip can be enhanced as the dimensions of the device are reduced; other physicochemical interfacial phenomena not usually encountered at macroscopic dimensions can also be exploited. Separation can, in addition, be carried out faster and more efficiently at smaller scales. Further, microfluidics offers integration capability such that the entire range of benchtop laboratory protocols from sample handling through reaction, separation, and detection can be incorporated and automated onto a single chip in a manner not unlike the unit operations of a chemical plant. With advances in large-scale mass microfabrication and nanofabrication, economies of scale can be capitalized to manufacture cheap, disposable, and portable handheld devices that could potentially revolutionize both personalized healthcare medicine through point-of-care diagnostics[4] or high-throughput drug discovery through massive parallelization.[5]

In the sections to follow, we provide an overview on the use of microfluidic devices for a number of applications in biology and biotechnology. Due to the vast nature of the subject matter and space limitations, our aim is not to provide a thorough and comprehensive review but to provide the non-specialist reader with a general and broad appreciation for how microfluidics can be used in each application. Given the large number of papers in this area, which has grown prolifically in recent years, it is impossible to be inclusive of even all of the key literature in each subfield—any omission, for which we assume sole and complete responsibility, is unintentional and we apologize to the respective authors for this. As there are also a number of excellent reviews on the basic fundamental physics governing microscale and nanoscale fluid phenomena,[6,7] we omit these altogether and proceed to discuss specific bioapplications that utilize microfluidic technology: gene manipulation and genomics (Section 2); protein manipulation and analysis (Section 3); cellular systems (Section 4); biochemical and pathogen detection (Section 5); high-throughput drug screening (Section 6); point-of-care diagnostics (Section 7); drug delivery (Section 8); and, biomaterials synthesis and tissue engineering (Section 9). Finally, we conclude with a discussion on the challenges faced in microfluidics and an outlook on what the future might hold.

2. Gene Manipulation and Analysis

While the sequencing of the 3 billion or so DNA base pairs and hence the identification of the 20 000–25 000 genes that make up the human genome has been completed through the Human Genome Project in 2003, there is still a need to develop new and more advanced sequencing and genotyping techniques, particularly to interpret such sequences and to apply this to improving the human condition or for forensic

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analysis. This includes determining the proteins that genes produce and their effect at the cellular level, which could unravel a deeper understanding of hereditary diseases and lead to the development of improved gene and drug therapy. In particular, the variation of DNA in gene sequences needs to be quantified and related to gene function. Efforts are already underway, for example, to detect single nucleotide polymorphisms (mutation of single base pairs at a specific DNA sequence within the genome), which are largely responsible for differences between individual human beings and hence the cornerstone of personalized medical care.

The Human Genome Project, which took 13 years to complete and cost around US$2.7 billion, nevertheless, underlines how expensive, laborious and time consuming the entire sequencing and genotyping process can be. The National Institutes of Health has since expressed its intent to reduce this cost to around US$1000 per complete genome sequence. For this to be realized, low-cost and efficient high-throughput methods for DNA sequencing are therefore required, for which microfluidics may provide the answer. In addition to DNA screening, microfluidics may also provide significant advantages over conventional laboratory protocols for other gene analysis and manipulation processes such as polymerase chain reaction (PCR) and DNA recombinant technology.

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2.1. DNA Microarrays

DNA microarrays[8,9] have been in existence even when microfluidic technology was still in its infancy and have since revolutionized the molecular biology landscape by offering the possibility for simultaneously interrogating a huge number of DNA by binding (hybridization) patterned arrays of DNA oligonucleotide probes immobilized on the substrate or gel with complementary nucleotide sequence targets, thus allowing fast monitoring of the expression of thousands of addressable genetic elements at the same time. Since its initial success in gene expression studies,[10] DNA microarrays have been employed for transcriptional profiling, genotyping, comparative genomic hybridization, and epigenetic analysis.[11]

The time required for the target analyte in the bulk to migrate toward the immobilized probes is however limited by the diffusion time scale, which is long compared to the kinetic time scales associated with molecular binding events. This is due to the low diffusivity of DNA ($D \sim 10^{-8}$–10^{-9} cm^2 s^{-1} depending on molecular length and conformation[12]); typical hybridization reactions therefore usually require hours, which is too long for high-throughput screening and portable field analysis. There are two primary ways that diffusion times, which scale as L^2/D wherein L denotes the diffusion length scale, can be shortened to minutes or even seconds using microfluidic technology. The first involves reducing the diffusion length scale by integrating the microarray within microfluidic channels in which the flow recirculates.[13–15] The large surface-area-to-volume ratio in microchannels as well as the reduction in sample volume allows for a larger number of oligonucleotides to be functionalized, thus increasing the target capture probability and hence the assay sensitivity.[16] The second involves overcoming diffusion limitations by enhancing long-range convective mixing.[17,18] A range of microfluidic actuation mechanisms exist to drive such convection, although most are typically ineffective due to the small Péclet numbers Pe (typically below 100) associated with conventional microfludic systems—the $Pe^{1/3}$ scaling for convective mass transfer enhancement then suggests that only a unit-order acceleration in the rate of mass transfer can be achieved.[19]

Electrokinetics remains the preferred method of microfluidic transport[20] and high-frequency surface acoustic waves (SAWs) may offer high-Péclet-number micromixing that is considerably faster than most other conventional means;[21] other uses of microfluidic transport in microarray formats are discussed in Section 3.1. Another advantage of electrokinetic and SAW microfluidic transport is that the transducer electrodes can be integrated on the device or chip, thus eliminating the necessity for peripheral fluid transfer and allowing for easier, more precise fluid control and handling compared to large and cumbersome capillary pumps.[20] In addition, the integration of microarrays into microfluidic channels permits the optimization of hybridization conditions, such as temperature, ionic strength, and denaturant concentration, upon demand to improve the specificity of an individual probe that is not afforded with conventional microarray technology. In addition to a reduction in the hybridization time, enhancements in sensitivity and spatial resolution have also been reported using microfluidic devices together with surface modification.[22]

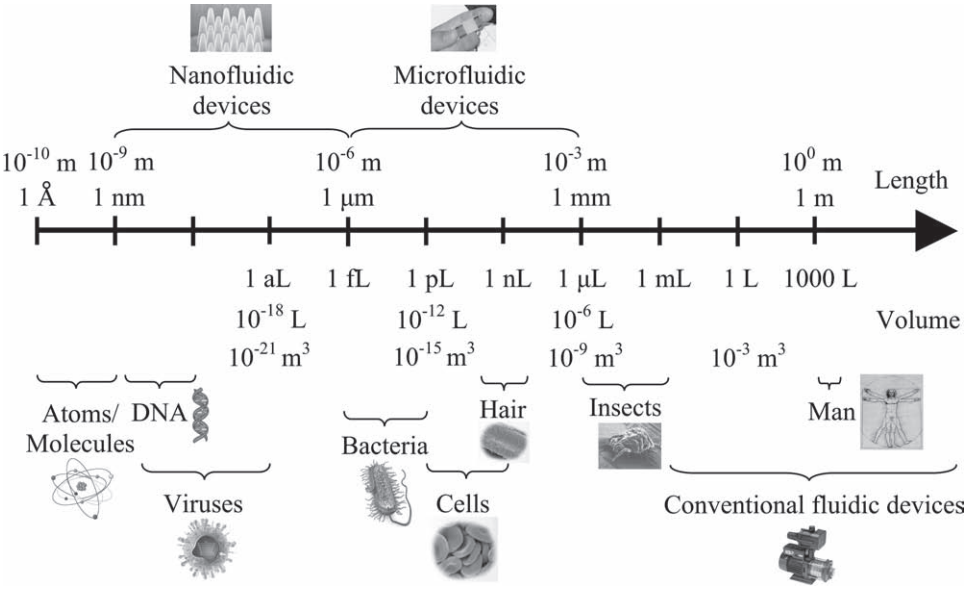

Figure 1. Characteristic length scales (approximate) of microfluidic and nanofluidic systems in relation to that of various biological entities.

2.2. Bead-Based Platforms for DNA Hybridization

More recently, there has been a drive towards the use of bead-based DNA hybridization and sequencing assays.[23–26] Silica, latex, and gold colloidal nanoparticles or quantum dots comprise a low-cost solid support with large surface area on which oligomers and fluorophores can be functionalized for the hybridization reaction[27]—compared to the 1 μm^2 capture area in a typical DNA microarray, even a 1 vol.% microbead suspension in a 100 μL sample provides an increase of 8 orders of magnitude in the capture area and hence a similar enhancement in the assay sensitivity.[19]

Unlike conventional DNA hybridization assays carried out on a 2D planar surface, the bead-based assay constitutes a 3D analogue, which facilitates more rapid and more efficient detection. Bead-based assays also offer the possibility for detection without the signal amplification typically required in planar formats due to the low fluorescence intensity afforded by the small area density of the surface immobilized antibodies and the high transmittance through the substrate. In addition, the presence of beads as obstacles dispersed in a convectively driven system disrupts the laminarity of the flow and hence enhances effective mixing in the sample. Further, recent advances in colloid chemistry has allowed multiple copies of oligonucleotide and fluorophore combinations to be functionalized onto a single bead.[28] Such a large library of fluorescent barcode tags then open up new and powerful possibilities for massively parallel, multitarget, and multiplex detection.

Nevertheless, the full potential of bead-based platforms can only be practically exploited if bead handling and manipulation mechanisms exist. Again, microfluidics can play a significant role here, for example, in identifying and sorting the beads after the hybridization has taken place. Paramagnetic beads have been used for this purpose but require the use of large quadrupole magnets due to their small magnetic moment, therefore rendering continuous flow in a microfluidic device difficult and costly.[29–31] **Figure 2** shows an integrated multiplex continuous flow microfluidic device for debris filtering and the sorting and trapping of colloidal beads[32,33] based on dielectrophoresis (DEP), which is the motion of particles under a non-uniform AC electric field. In DEP, such bead manipulation is possible due to the reversal of the polarization along the surface across a crossover frequency that is dependent on bead size, shape, and dielectric properties.[20,34] While the 100-bead-per-second sorting speed

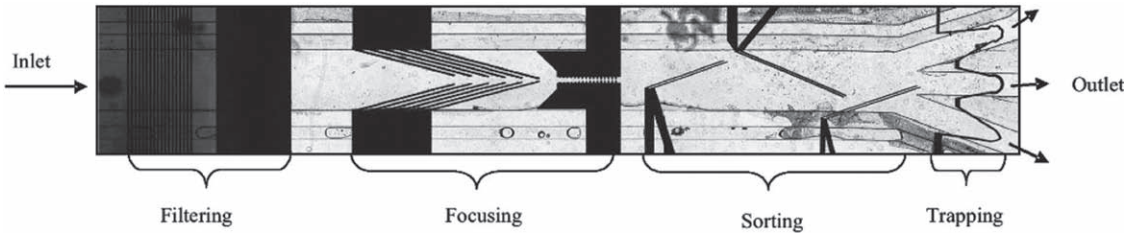

Figure 2. Integrated multiplex continuous flow microfluidic device for dielectrophoretic sorting and trapping of colloidal beads. Reproduced with permission.[32] Copyright 2007 American Institute of Physics.

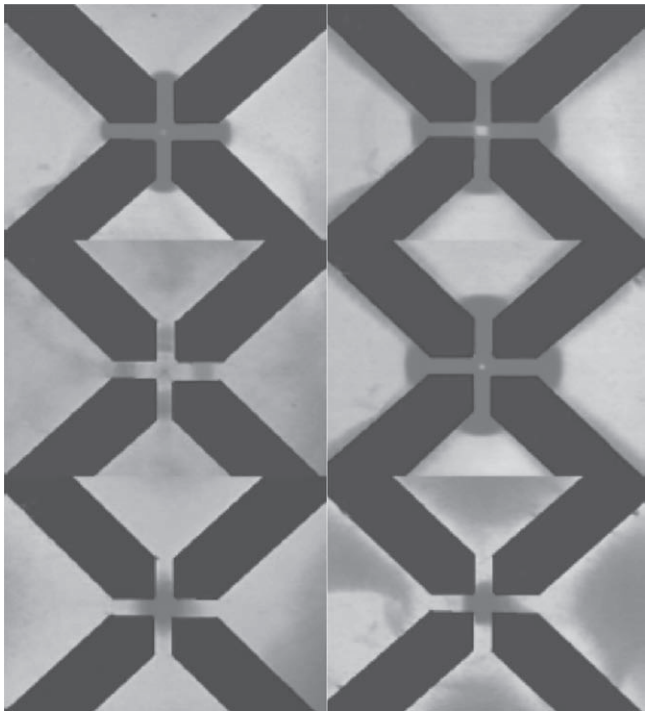

Figure 3. DNA identification within 10 min using quadrupole electrodes to trap silica nanocolloids functionalized with oligonucleotides specific to certain target DNA sequences. The frequencies in each row are 500, 1.5, and 2.3 MHz from top to bottom, respectively. The two columns from left to right show the hybridized beads and those in a strong electrolyte buffer solution, respectively.[19,20]

is still two orders of magnitude smaller than that afforded by flow cytometry, the device offers tremendous possibilities as a low-cost disposable and portable chip-scale diagnostic tool.

The crossover frequency and the effective bead hydrodynamic radius also has a strong dependence on the DNA concentration and conformation of the hybridized DNA, thus allowing enhanced calibration precision of the number of captured DNAs.[35] The possibility of DNA identification within 10 min is shown in **Figure 3** wherein silica colloidal beads functionalized with oligonucleotides specific to certain target DNA sequences are trapped using a quadrupole electrode. In fact, the sensitivity of the crossover frequency to the hybridization removes the necessity for laborious probing of individual colloids using sophisticated fluorescent imaging techniques since it permits macroscopic imaging of colloidal suspension patterns.[20]

As an alternative to microspherical solid colloidal beads, it is also possible to exploit the advantages associated with the large surface area, tunable surface chemistry, electrochemical characteristics, and biocompatibility of carbon nanotubes (CNTs). In addition, the absorption and hybridization of DNA onto CNTs can significantly enhance the electron transfer rate.[36] High-sensitivity DNA detection has therefore been reported using electrochemical impedance sensing with CNT electrodes (**Figure 4**).[36,37] The use of nanoporous micron diameter agarose beads within which capture antibodies are immobilized onto the surface of the mesh fiber network has also been proposed to increase immunoassay dimensionality and capture antibody density.[38] Individually embedded within millimeter-sized well arrays in a plate, these bead-based biosensors constitute a novel multiscale approach and have been shown to produce a superior detection range and sensitivity compared to conventional 2D planar enzyme-linked immunosorbent assays (ELISA). The 280 μm beads are however typically too large for microfluidic systems and are more suited to microarrays, although it can be envisaged that smaller beads that can be dispersed in an analyte suspension can further increase the speed and sensitivity of detection. Antibody loading and antigen transport through the 100 nm order pores are diffusion-limited, although one possibility is to use nanoporous electrokinetic transport to overcome this limitation.[39]

Microfluidic actuation can also be harnessed to enhance the speed and sensitivity of bead-based assays. Microcentrifugation driven by electrohydrodynamic[40–44] or acoustic[45–47] means can be used to rapidly concentrate the beads (**Figure 5**) to achieve an enhancement in detection sensitivity and to lower detection limits or to enhance device specificity by concentrating desired targets near the probes.[19]

2.3. On-Chip Electrophoretic Separation for Gene Analysis

The enormous popularity of genetic analysis based on electrophoretic separations historically stems from the significant advances achieved in the early 1990's with capillary electrophoresis (CE),[48] and subsequently capillary array electrophoresis (CAE),[49] systems. These eventually became the standard in DNA electrophoresis, effectively replacing the original slab gel electrophoresis systems originally used when the Human Genome Project first commenced. Given that microfluidic technology owes its existence, at least in part, to CE technology, it would therefore seem logical that a large focus of current efforts to develop miniature high-throughput genetic analysis systems is based on integrating the microfluidic sample handling platforms with advances in CE and CAE technology.[50] Here, we discuss a number of integrated devices that have been developed for genetic analysis based on electrophoretic separations.

2.3.1. DNA Sequencing Using the Sanger Method

Despite the introduction of more recent sequencing technologies, the dideoxy chain termination method of Sanger,[51] which interrupts the enzymatic replication and fragments the base DNA sequence into a ladder of DNA segments of differing lengths that is then electrophoretically separated, has remained the most commonly used and the only viable method for the de novo sequencing of large new genomes or the sequencing of highly rearranged genome segments such as that in chromosomal regions that display structural variations. This is because of its robustness, low error rate, and long read lengths (around 700–800 base pairs); alternative technologies such as pyrosequencing and massively parallel signature sequencing, on the other hand, sacrifice read lengths to achieve gains in low costs and high throughput.[52]

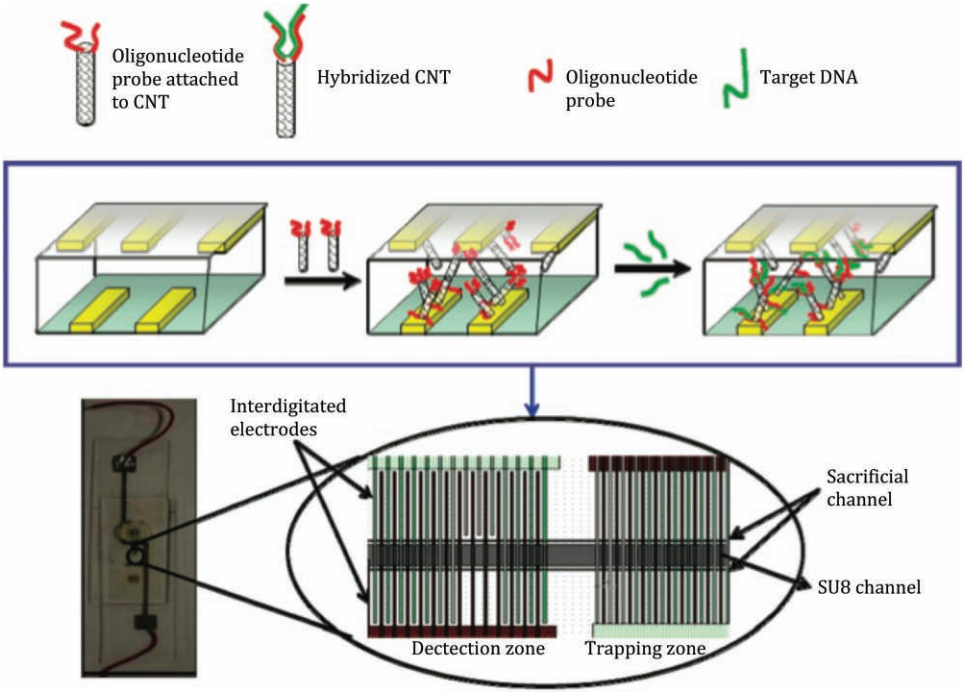

Figure 4. Schematic depiction and corresponding image of the open-flow microfluidic carbon nanotube impedance sensing platform for DNA hybridization. The inset shows a magnification of the electrodes and channels. Reproduced with permission.[36] Copyright 2009 American Chemical Society.

One of the distinct advantages of the Sanger method is its potential for miniaturization using micro/nanofabrication and integration with sample preparation strategies through the incorporation of microfluidic technology. Indeed, the state of the art has advanced considerably since the development of the first 12-lane parallel DNA separation microdevice using CAE in 1997.[53] Advances in microtechnology has allowed smaller and denser microchannel arrays with complex turn geometries to be fabricated such that 96- (**Figure 6**),[54] 384-,[55] and even 768-lane[56] CAE devices are now routinely demonstrated for multiplex sequencing.

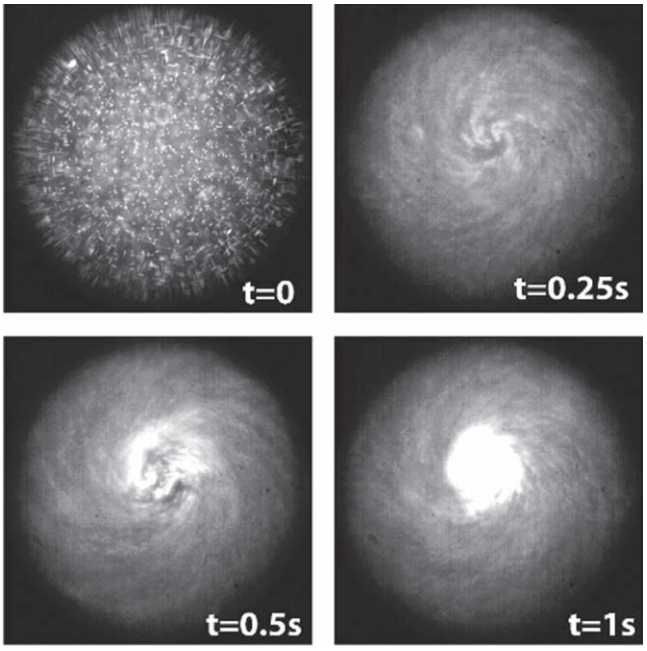

Figure 5. Intense inertial microcentrifugation of 500 nm fluorescent particles in a 0.5 µL fluid drop driven by surface acoustic waves for rapid particle concentration and separation. Reproduced with permission.[47] Copyright 2008 American Institute of Physics.

Nevertheless, the beauty of such advances does not just lie in fast and parallel processing, but also in the ability to incorporate two other ancillary procedures required in the Sanger method, i.e., thermal cycling and sample purification, with the electrophoretic separation within a microfluidic device.[57] A number of other miniaturized electrophoretic devices for DNA analysis are also reviewed in various publications;[58–60] for a discussion on associated fabrication technology and surface modification chemistry, see Kan et al.[61] Issues surrounding efficient inline methods to achieve nanoliter sample injection, purification, and preconcentration without introducing hydrodynamic dispersion (band broadening) to deliver the required sensitivity, reliability, and reproducibility in an integrated microfluidic device, however, are challenges that still need to be addressed in microfluidic devices. Another thrust of recent research has been on alternatives to circumvent the need for biased reptation (use of gel and polymer matrices) in DNA electrophoretic separation due to the size-independence of the electrophoretic mobility (ratio of charge to the drag coefficient), which poses a barrier to high throughput and high efficiency compared to if the separation can be conducted in free solution. One possibility, known as end-labeled free-solution electrophoresis, is to label the ends of the DNA with large uncharged molecules to provide

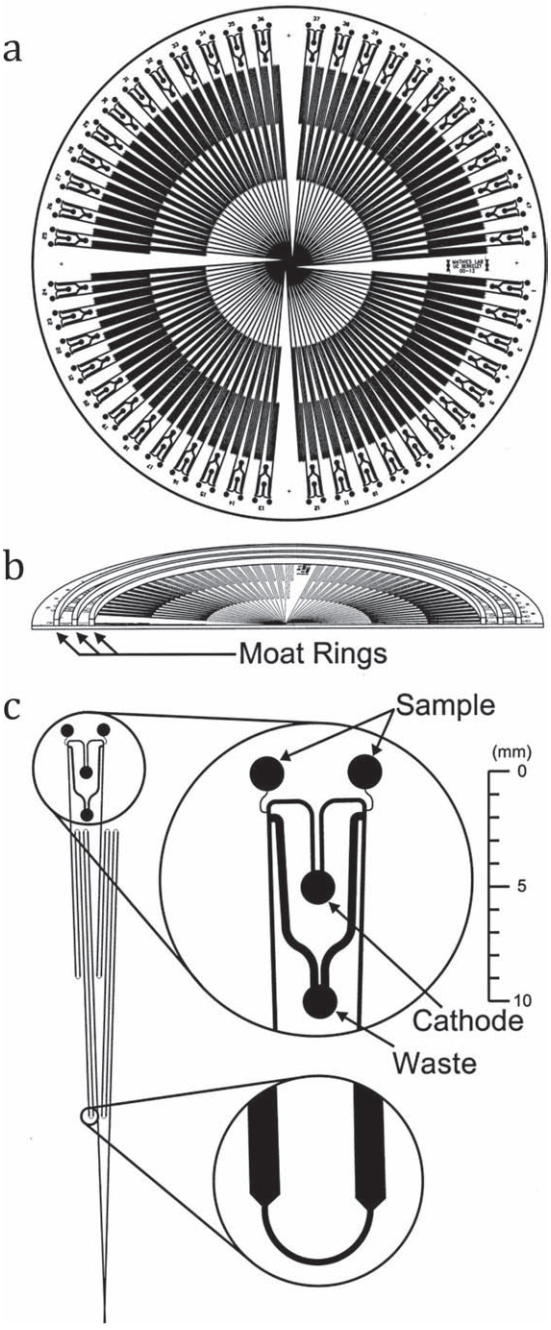

Figure 6. DNA sequencing in a 96-lane capillary array electrophoresis platform. The plate is shown in (a) and the cut-out in (b). A schematic of an individual lane pair is shown in (c), the inset of which shows a magnification of the sample injection, buffer and waste ports, and the turn channel. Reproduced with permission.[54] Copyright 2002 National Academy of Sciences, USA.

sufficient drag on the DNA chain such that the electrophoretic mobility is endowed with some size-dependency.[20,62]

A recent promising development based on the Sanger approach, which has the advantage of low cost, ease-of-use, and most importantly, short sequencing runs such that the entire device can be integrated into a chip, is large-scale DNA sequencing by denaturation, in which Sanger fragments of different lengths labeled fluorescently according to their end base type, achieved by the insertion of fluorescently tagged nucleotides during replication, are sequentially denatured by heating. Since the melting temperature of short fragments correlates with the number of bases, the base sequence of the target can then be determined by analyzing the decrease in the fluorescence signal.[63]

2.3.2. Genotyping

While 99.5% of DNA sequences between two individuals are identical, any variation in the genome present in less than the remainder of this percentage of the human population (mutations) could potentially result in a high risk of genetic diseases (e.g., cystic fibrosis, sickle cell anaemia, etc.). Even when the genetic variation naturally occurs in more than about 0.5% (polymorphisms), this information could be useful in understanding how humans respond to pathogens or drugs and therefore in mapping a strategy for personalized healthcare development. As a consequence, and with the completed mapping of the human genome, efforts have turned to identifying genetic variation through the International HapMap Project.

There are a number of ways based on electrophoretic separation in which sequence variation in DNA can be detected. One of the most popular methods is to detect insertions, deletions, or substitutions in a single base pair, known as single nucleotide polymorphism (SNP), which occur at a probability of about 1 in every 1000 base pairs. Because most current SNP detection devices involve expensive DNA hybridization microarrays,[8,9] which are limited by the large number of synthetic probes required, and also because prior knowledge of the sequence of interest is required,[61] there has been recent interest in using microfluidic platforms to carry out SNP detection[64] (although microfludics itself can also be used to enhance the performance of these DNA microarrays through convective mixing;[65,66] see also Section 2.1). Most of these involve the use of an electrophoretic-separation-based technique known as restriction fragment length polymorphism (RFLP) wherein sequence-specific primers and restriction enzymes are used to recognize the sites where the SNP of interest occurs by cleaving the DNA fragments on either side of these sites.[67–69] An example of a low-cost microdevice which combines polymerase chain reaction (PCR; see Section 2.4) to amplify the DNA strands by integrating heaters, temperature sensors and control valves into the device with gel electrophoresis to carry out RFLP to identify an influenza viral strain is shown in **Figure 7**.[70]

Another electrophoresis-based method which discriminates single base pair differences through electrophoretic mobility variation in the 3D conformation of fragmented single-strand DNA molecules is single-strand conformation polymorphism (SSCP).[64] This was demonstrated for the detection of common mutations in breast cancer susceptibility genes within 120 s.[71] High-throughput SSCP analysis has also been shown with a 384-lane CAE.[72] Alternatively, SSCP can be combined with heteroduplex analysis (HA),[73] which is based on the difference in the electrophoretic migration speeds between the heteroduplex form of double-stranded DNA, which migrate

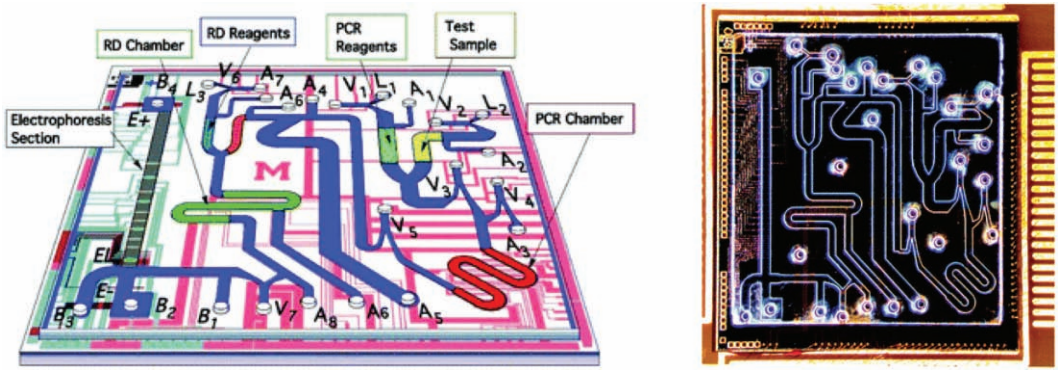

Figure 7. Schematic (left) and image (right) of a microfluidic device that integrates sample handling and manipulation, DNA amplification using PCR, and separation via gel electrophoresis for restriction fragment length polymorphism analysis for viral strain subtyping. Reproduced with permission.[70] Copyright 2005 The Royal Society of Chemistry.

slower due to their open configuration around mismatched bases, and the corresponding homoduplex;[64] a microfluidic SSCP-HA device has also been fabricated to analyze common gene mutations associated with hereditary haemochromatosis.[74]

A bead-based platform for picomolar-sensitive DNA hybridization (Section 2.2) has also been developed for SNP detection in a minute without requiring repeated sample rinsing, washing, and heating.[75] The 500 nm silica beads were functionalized with species-specific oligonucleotide probe sequences (26-bases) which are trapped at the electrodes using DEP. As the DNA solution is flowed through and over the electrodes, unbound oligonucleotide probes or non-specific DNA molecules can be removed by the shear flow, thus allowing high detection specificity in the hybridization event between the target DNA and single mismatch probe sequences localized at the electrodes, captured through fluorescence microscopy.

The need to analyze DNA that has been degraded or DNA in low copy numbers in forensics has also spurred activity in the development of rapid and high-throughput devices for short tandem repeat (STR) typing.[76] STRs are highly polymorphic genetic variations occurring in short sequences repeated throughout the genome. Both CE and CAE have been successfully combined with PCR for STR analysis,[77,78] although it is also necessary for other sample handling steps such as sample preconcentration as well as post-PCR cleanup and separation to be integrated[59]—this was recently achieved using streptavidin-modified photopolymerized capture gel chemistry.[79]

2.4. Polymerase Chain Reaction (PCR) and DNA Recombinant Technology

It has been seen above that PCR, which facilitates the amplification of DNA template (oligomer) copies such that a sufficient number is acquired for subsequent analysis, is an important integral step in genomic analysis. It is therefore unsurprising that considerable attention has been paid to developing microscale PCR methods given its importance in the effort toward the development of miniaturized genetic analysis systems.[80] Here, we refrain from a lengthy discussion on microfluidic PCR technology and refer the reader to various reviews on the subject.[81–83] Generally, PCR microfluidic devices either adopt a stationary configuration wherein the sample is held in a microchamber and the temperature of the chamber is cycled (or the sample transferred through sequential microchambers held at different thermal conditions) as in conventional PCR, or a flow-through configuration[84,85] wherein the sample is pumped either straight through or cycled back and forth different thermal zones (each responsible for a particular process, i.e., denaturation at around 94 °C to separate the double-stranded DNA into individual strands, annealing at approximately 55 °C to rehybridize or bind the primer to the single-strand template, and extension at around 72 °C to elongate the single-stranded DNA; each temperature cycle approximately doubles the number of DNA template copies). PCR has also been carried out by convecting the fluid through different temperature zones wherein Rayleigh–Bénard convective cells are established.[86,87] More recent efforts include integrating PCR with sample preparation steps such as cell isolation and DNA purification,[88] as well as DNA or even whole cell analysis.[89,90]

Microfluidic systems have also been developed for DNA recombinant technology, which can be exploited for the production of synthetic insulin or the insertion of genes into plasmid DNA for gene delivery. In DNA recombinant technology, several steps are required, including gene isolation, purification, ligation, and transformation, although a complete device that integrates all of these steps to carry out the entire DNA recombinant process on a chip has yet to be realized. Nevertheless, significant progress has been made in developing on-chip systems for each of these steps. For example, microfluidic devices fabricated from multilayer soft lithography[91,92] (**Figure 8**) have been demonstrated for isolating mammalian cell RNA and bacterial cell DNA, which also includes integrated cell loading and lysis as well as target purification and recovery.[93,94] Similar fabrication processes were also used to manufacture a chip-based gene ligation procedure, in which the enzyme ligase is employed to link a linear target DNA to a plasmid.[95]

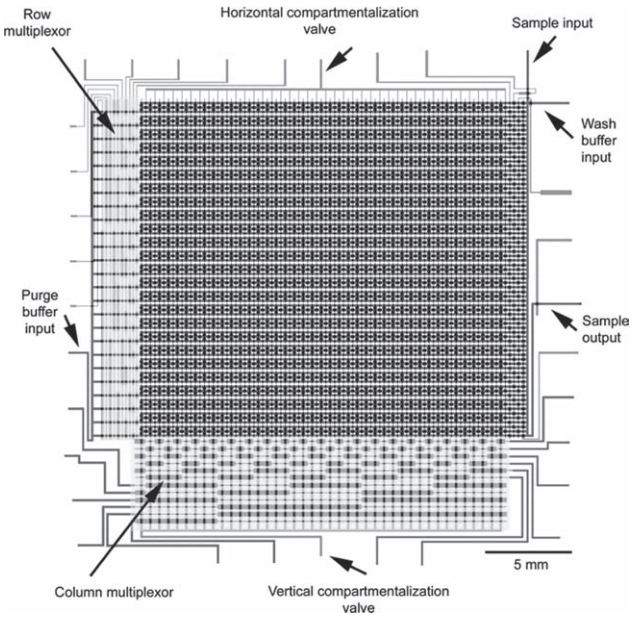

Figure 8. 25 × 40 array of individually addressable 250 pL microchambers housed in a microfluidic device for large-scale integration. Connecting these fluidic ports are a network of valves fabricated using multilayer soft lithography. Reproduced with permission.[92] Copyright 2002, American Association for the Advancement of Science (AAAS).

2.5. Emerging Technologies for Microfluidic Genetic Analysis

Currently, a host of novel technologies are being developed to carry out genetic analysis. An area that has recently received significant attention is nanofluidic research, wherein the ability to transport single molecules through nanopores offers considerable promise for DNA sizing, separation, sequencing, and sensing without requiring fluorescent labeling.[96] For example, the size measurement and subsequent separation of DNA fragments has been demonstrated using entropic traps wherein constrictions with dimensions smaller than the free solution DNA's radius of gyration were employed, the rate of DNA entry being dependent on the fragment length.[97,98] Using restriction enzymes, the elongated DNA in the nanochannel can also be cut at specific sequence locations.[99]

Arguably, the most efficient way to drive single-molecule transport and separation through nanopores and nanochannels is to use electrokinetics,[20,39,100,101] given that individual nucleotides can be distinguished by the amount of ionic current they modulate as they flow through the nanopore.[102,103] Electrokinetically driven nanopore or nanochannel transport[39,104,105] can also be coupled to the bead-based platforms discussed in Section 2.2—the focusing of the electric field within the nanochannel produces a high-field region that can selectively attract (or repel) nanobeads exhibiting positive (or negative) DEP. Moreover, bead concentration within the nanochannel can be detected quite sensitively and subsequently quantified using electrical impedance measurements, much like Coulter counters, because of the field focusing and because the beads resemble large macroscopic ions with an abundance of mobile counter-ions that can drastically increase the local conductance of the solution. In effect, the oligonucleotide-functionalized beads that exhibit positive DEP after hybridization of the molecular target and that are hence attracted to the nanochannel impedance sensor can act as impedance-based detection reporters for the hybridization event. Such nanochannels are ion selective and can deplete or enrich ions at their entrance[104,105] in a similar manner to membranes and can enhance the impedance signal of nanocolloids or hybridized molecules as well as promote larger hybridization yields at an optimum pH. The depleted region can also produce an extended space charge region that is capable of sustaining strong microscale-induced charge electro-osmotic vortices, which can be exploited to concentrate molecules more rapidly than convection, and more interestingly, to shear off nonspecifically bound molecules;[104] such shear-endowed selectivity has been demonstrated to yield single mismatch discrimination in Section 2.3.2.[75] An integrated nanobead/nanochannel platform therefore offers the advantage of fast detection times as well as high sensitivity and selectivity. The impedance detection approach can also eliminate the need for fluorescent labeling and optical detection, which is cumbersome and prohibitive to miniaturization of the complete platform. Consequently, we anticipate that multitarget multitasking platforms based on such integrated nanobead/nanochannel technologies will pave the way for the next generation of chip-based biosensors in the near future.

A novel and promising technology for DNA diagnostics using electrospraying (or electrohydrodynamic atomization; see also Section 8.2.1) has also been recently demonstrated.[106,107] Here, target single-stranded DNA is used as a linker for two nanocolloids with different oligonucleotides complementary to two ends of the target DNA. Consequently, the presence of the target DNA converts monomer nanocolloids into dimers. If the target DNA is sufficiently large, hybridization onto a single nanocolloid can alter the effective size of the nanocolloid. As such, a bimodal distribution of nanocolloid sizes exists in solution in the presence of the target DNA. If this binary solution is ejected from a DC Taylor cone meniscus during electrospraying, the nanocolloids of varying sizes are observed to occupy different conical plumes and hence assume different flight trajectories. The net result is that hybridized or linked nanocolloids deposit on rings of different radii on the target substrate compared to the unhybridized nanocolloids, as observed in the inset (top) of **Figure 9**. The number of nanocolloids deposited in each ring can then be quantified using a picoammeter, as the nanocolloids carry charge that is proportional to their size. Given that the electric field and shear are high within the Taylor cone, the target DNA is elongated to facilitate faster hybridization onto the colloids—hybridization detection is then possible in under 5 min if the nanocolloids were to be mixed with the sample solution and simultaneously electrosprayed.

3. Protein Manipulation and Analysis

The success of genomic screening has opened up new potential applications for microfluidic systems particularly

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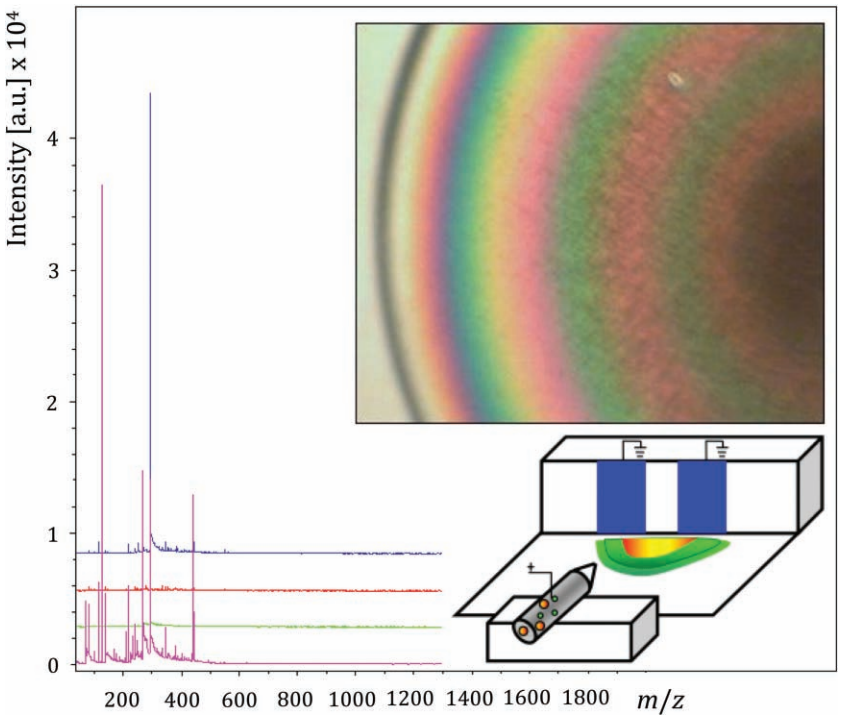

Figure 9. MALDI mass spectra of the electrospray-deposited products of *E. coli* cell lysis. Both cell lysis and separation were carried out simultaneously in the electrospray prior to deposition onto the substrate, following which MALDI was performed. The inset shows the ring patterns of the electrospray deposits under white light scattering (top) and a schematic of the experimental setup of the electrospray (bottom). Reproduced with permission.[107] Copyright 2009 Institute of Physics.

in biochemical analysis which cannot be carried out at the DNA level—for example, insight into signal transduction, cell differentiation, receptor activation, and malignant transformation cannot be gleaned from DNA sequence data and hence protein expression and interaction as well as post-translational modification must be studied at protein level.[108] Using microfluidics for proteomics, however, is by far a greater challenge compared to its genomic counterpart. To start with, there are considerably more proteins than genes, perhaps between 300 000 to several millions,[109] assuming that each gene can produce several proteins; protein sizes can also range from simply tens of amino acids in toxins to 27 000 in titins. Moreover, protein samples are often limited, but unlike DNA, there is no PCR analogue for proteins and hence a microfluidic system for proteome analysis has to be extremely sensitive. Poor peptide stability is also another contributing factor.

The majority of work carried out on microfluidic proteomic systems to date has therefore focused on attempts to integrate other steps such as flow-through sampling and sample preparation and enrichment, as well as to interface mass spectrometry with chip-based liquid chromatography for simultaneous protein separation and detection. Efforts are also being conducted to miniaturize and integrate the mass spectrometer—there has been steady progress in recent years in scaling down ion trap and quadrupole mass analyzers,[110] although the complexity of the tandem mass spectrometer poses a significant challenge for its reduction onto a chip-scale device.[111] In addition, microfluidic processes are also currently being developed for other processes such as protein crystallization.[112]

3.1. Protein Chip Microarrays

In a manner similar to their DNA analogues, microarrays have been developed for high throughput protein analysis. Again, these microarray formats do not necessarily incorporate microfluidic technologies although microfluidics can not only play a key role in enhancing convective mixing, as discussed in Section 2.1, but also in peripheral fluid handling and transfer onto the chip as well as integrated on-chip fluid transport and handling.[113] For example, electrosprays,[114,115] surface vibration[116] or other contact printing methods[117] can be used to create protein spots or even whole live cells[118] in the array rapidly and quickly without requiring physical or chemical templating on the chip substrate; protein denaturing during these print processes can be minimized through the use of high-frequency fields above that of the inverse molecular relaxation timescale (i.e., 100 kHz and above), such that there is insufficient time in each half cycle for molecular shearing to occur.[119–123]

Alternatively, microarrays can be manufactured with the aid of electrowetting[20,124–126] or acoustic droplet transport[21,127] to precisely and controllably move sample droplets into position in the arrays. Electric or acoustic fields can also accelerate evaporation of the positioned sample droplets into dry protein spots with sizes smaller than that currently possible with individual wet-wells. Not only are proteins more stable in dry crystalline form, an array of multiple protein stains can constitute miniaturized libraries which can be subsequently dissolved and retrieved; the small sizes of these protein stains allow for higher throughput than that currently afforded with even 1536 wet-well microarray trays.[128] Nanoarray technology is also currently being developed as a novel method to graft DNA-protein conjugates onto a surface.[129]

In general, protein microarrays exist in two forms.[130] Functional protein microarrays consist of full-length proteins that are immobilized onto the chip and are used to investigate the interaction of protein and other biomolecules, primarily in the identification of potential drug candidates. On the other hand, analytical microarrays, which consist of an array of antibodies over which proteins are probed, are generally employed for monitoring protein expression and diagnostics.

Protein microarrays, nevertheless, possess certain inherent limitations that may render a multiplex-affinity

assay ineffective. For example, poor sample preparation often leads to irreproducible results and protein heterogeneity possessing different solubilization and affinity requirements restricts the assay to a small number of similar proteins.[131] Precise and controllable microfluidic sample handling procedures and protein digestion procedures are possible ways to circumvent these limitations. In the latter approach, presample microfluidic enzymatic digestion (see Section 3.3) to break down the proteins into peptides, which are more stable than proteins and can be more readily detected with mass spectrometry, could be employed. Alternatively, the native peptide form can be analyzed in microarray format without involving protein digestion processes, an approach known as peptidomics.[131]

3.2. Mass Spectrometry Interfacing

Even with the advances in detection and characterization methods, mass spectrometry (MS) remains a central tool, especially for proteomics, given its accuracy and speed. As MS separation and detection is based on the mass-to-charge ratio of gas phase ions, and until mininaturized MS systems can be embedded and integrated into microfluidic devices, a robust method for coupling the on-chip separation and other ancillary operations with the mass spectrometer is required. Fundamentally, chip-MS interfaces are centered around the technology associated with the ionization technique employed, the two most common being electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).[132,133]

3.2.1. MALDI-MS Interfacing

While MALDI-MS is fast and efficient, allows for high-density processing, and can tolerate the presence of impurities, contaminants and salts to a certain extent, the necessity for a laser requires fine tuning of optical properties in order to ensure quality and reproducibility in the results; signals are thus dependent on the laser wavelength, pulse energy as well as the time between the pulses and the impact angle. The small laser spot size relative to the sample also necessitates multiple laser pulses targeted at different sample regions in order to obtain a statistical average of the local concentration within the sample.[111] Moreover, online coupling MALDI with MS (usually time-of-flight mass spectrometry) is difficult as the separated analytes first need to be deposited onto a target plate to be ionized inside the vacuum source of the mass spectrometer. In offline MALDI, samples still have to be directly spotted from the chip onto the matrix-coated target, but the microfluidic sample processing is completely decoupled from MS analysis and hence the MALDI process can be carried out later.[134,135]

In this case, direct printing of separated analytes onto the target, either by mechanical (e.g., piezoelectric),[108,136] electrical (electrospray)[123,137] or acoustic[116,138,139] means is commonly employed. This can be coupled with the front end of a microfluidic device for sample pretreatment and an enzyme microreactor for proteolysis (see Section 3.3), as illustrated in **Figure 10**.[108] Nevertheless, precise volume control of the droplets can be difficult with direct printing, and, in the case of microchannel arrays that are closely separated, interference between droplets or between adjacent channels could occur.[134] Alternatively, electrowetting has also been used to combine sample preparation and purification wherein the sample and matrix droplets are moved, mixed, and deposited on a surface, which is then used as the target substrate.[140]

Online microfluidic MALDI-MS coupling can be achieved using continuous through-flow to transport the analyte sample within a liquid matrix, which is then delivered to

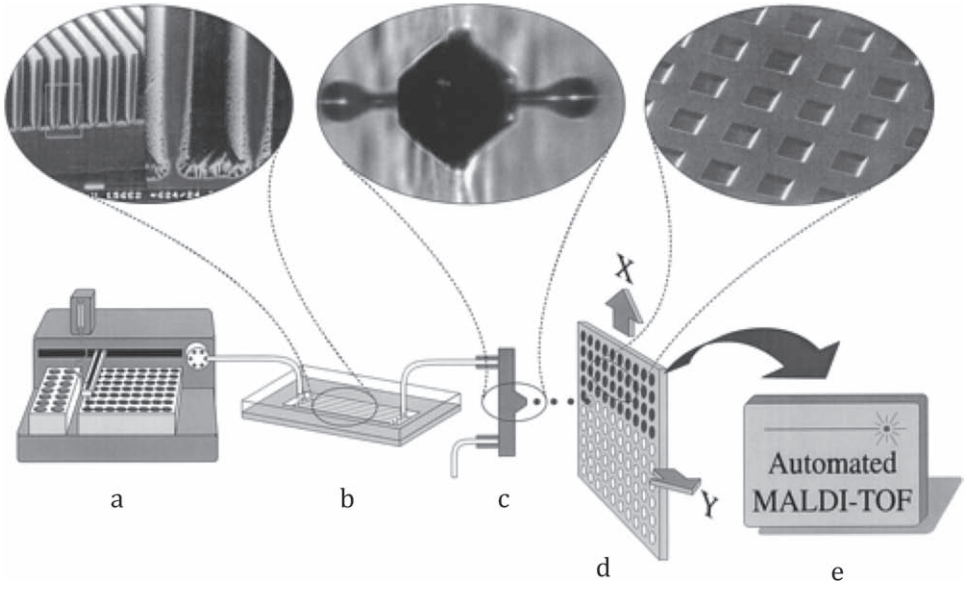

Figure 10. Integrated microfluidic system for the identification of proteins incorporating a) sample injection and pretreatment, b) enzyme digestion, and c) sample dispensing onto d) microvials for subsequent detection via e) MALDI-time-of-flight MS. Reproduced with permission.[108] Copyright 2000 American Chemical Society.

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a point where the laser beam is focused. Either the vacuum within the mass spectrometer or the deposition methods discussed above for offline microfluidic MALDI-MS interfacing can be used to deliver the fluid to the ion source.[141,142] For heterogeneous samples with large biological molecules, the MALDI sample, however, is often too complex to allow identification of individual molecules. A promising way to presort the large biomolecules is to spray them into different rings, as was carried out on the lysed and separated products of *Escherichia coli* as shown in Figure 9; note that both cell lysis and subsequent separation were performed within the electrospray itself prior to spray deposition and MALDI. As seen in the inset, lysed biomolecules from the cell are shown to be sorted into different rings; the resulting MALDI-MS spectrum of each ring then yields different molecular fingerprints.

3.2.2. ESI-MS Interfacing

Online coupling between microfluidic separation chips with MS is more straightforward using an ESI-MS interface given the comparability between the typical nano- or microliter-per-minute microchannel flow rates with those used in electrospraying and since the electrospray facilitates through-flow from the chip to the spray orifice. While there are various designs that have been proposed, these fall into three broad approaches based on the nature of the electrospray emitter used: blunt edge, transfer capillary attachment, and emitter tip;[111] most recent designs fall in the latter two categories.

The original miniaturized on-chip electrospray designs consisted of a planar glass microchip etched channel that culminated in an open end which functioned as the electrospray orifice.[143] A subsequent high-throughput version simply consisted of parallel multichannels, each with an open end.[144] Although simple in design, the blunt edge chips suffered from several limitations. The flow needed to be pressure-driven as the currents associated with electrospraying were insufficient to generate electro-osmotic flow. Difficulties with regulating flow perturbations also resulted in meniscus ballooning at the orifice, disrupting spray stability and leading to the formation of dead volumes, which then introduced hydrodynamic dispersion and hence sample band broadening. The problem can be remedied by incorporating a gas sheath flow around the electrospray meniscus by coupling an integrated nebulizer to a sub-atmospheric liquid junction but this generally causes dilution of the sample and the introduction of background noise, both of which result in a compromise in the detection sensitivity.[145]

In the second category, a high voltage is applied to a transfer capillary attached to the edge of a microchannel that acts as the electrospray interface. This allowed the orifice to be smaller in dimension to that of the blunt edge, thus increasing the stability and hence the spray performance. The capillary attachment setup is, however, difficult to control and does not facilitate good reproducibility. To circumvent these issues, the transfer capillary can also incorporate a similar sheath flow and liquid junction to the blunt edge devices. Sheathless interfaces have also been developed to eliminate the dilution effect.[146] To prevent electrolytic reactions which cause bubbles to form, the electrical connection can take the form of a Nafion conducting polymer membrane which acts as a charge-selective salt bridge, thus permitting current penetration through the membrane.[147]

With the ability to cheaply fabricate emitter tips and to attach them onto a microchip, high throughput can be achieved through direct electrospraying with multiple parallel arrays of microfabricated emitter tips (over 100 in number).[148] Recent advances in microfabrication has enabled these tips to be embedded at the end of the microchannel at the edge of the microfluidic device (**Figure 11**).[149]

3.2.3. Challenges and Future Technology

Despite the relative maturity of the technology and the availability of commercial high-performance liquid chromatography (HPLC) ESI-MS microfluidic chips on the market,[150] several obstacles remain before a robust, optimized, cost-effective, high-throughput microfluidic device providing flexibility for coupling to different MS configurations can be achieved. In particular, design problems related to electrospray stability, ionization efficiency, detection sensitivity, separation efficiency, speed, accuracy, spectra quality as well as sample loss and contamination need to be addressed.[111]

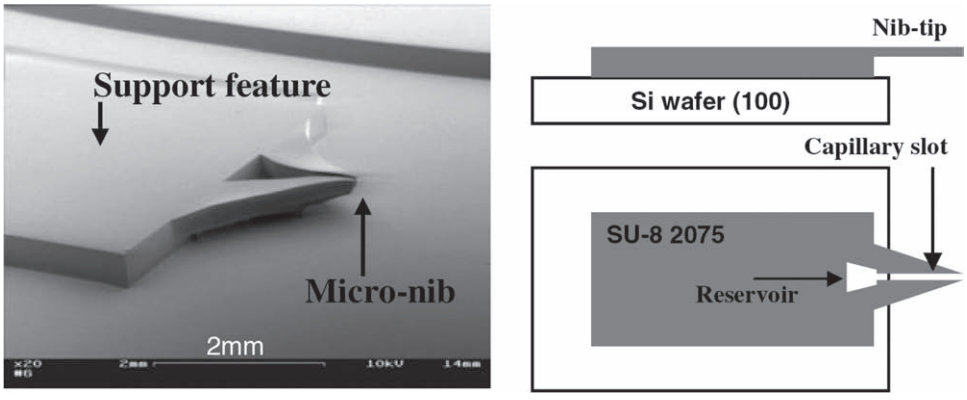

Figure 11. Scanning electron microscope image (left) and schematic (right) of the nib-like emitter tip of an ESI-MS interface fabricated in SU-8 epoxy using double exposure lithography. Reproduced with permission.[149] Copyright 2004 Institute of Physics.

A promising technology that has recently emerged for chip-MS coupling is SAW atomization.[151] In particular, SAW atomization was demonstrated for MS detection of peptides—these were transferred to the gas phase through the atomization process, following which they fragmented under a collision-driven dissociation process.[152] There are several advantages of using SAW for MS. The spectra produced using SAW atomization did not lead to contamination of matrix ions at low mass-to-charge ratios unlike with MALDI. Moreover, the SAW-MS coupling can be carried out on planar devices without the need for capillaries, reservoirs, or electrode-contact.

Further, there has recently been growing interest in conducting microfluidics on paper, wherein virtual microchannels and zones consisting of hydrophilic regions patterned along hydrophobic barriers are formed in a paper substrate to carry out a variety of diagnostics at low cost (see Section 7).[153,154] Using SAWs, it is possible to imbibe the fluid and whatever analyte that is contained within it from the paper and to subsequently atomize it to form a monodispersed distribution of aerosol droplets containing the analyte. In particular, the extraction of proteins from paper was demonstrated, with the post-atomized sample showing little evidence of molecular degradation.[122]

3.3. Sample Preparation & Pretreatment

While miniaturization of protein analysis systems carries with it tremendous benefits, a microfluidic device is only truly miniature, cost-effective, and practically useful for screening large numbers of proteins if all other ancillary processes associated with the analysis in addition to mass spectrometry interfacing is incorporated onto the device. This includes integrating all sample handling (e.g., dispensing and injection[108,155]), purification, enrichment, preparation, and pretreatment procedures onto the microfluidic device. A broader discussion on microfluidic sample preparation, which includes filtration/separation (e.g., microdialysis or liquid–liquid extraction), sample preconcentration (e.g., by field-amplified stacking or isotachophoresis) and analyte derivatization (e.g., specific or nonspecific molecular labeling for subsequent detection), can be found in various reviews on the subject;[156,157] here, we will restrict our short discussion to certain specific steps in microscale protein analysis.[158,159]

Purification of protein samples generally involves the removal of salts and buffers.[133] An example of an attempt to incorporate on-chip sample purification into a chip-based ESI-MS interface is a desalting procedure involving the use of membranes to capture target analytes with water being used to rinse off the salts.[160] Another example moves sample drops using electrowetting[20,125,161] to deposit the protein at specific locations on the microfluidic chip over which a water drop is moved to dissolve and remove the impurities, following which a solvent drop is dispensed and dried to form a matrix for subsequent MALDI-MS analysis.[162]

Sample enrichment steps are often necessary, as a small number of proteins exist in abundance in blood plasma, thereby making the detection of other proteins that exist at low concentrations difficult. For example, the concentration of albumin in plasma is often 10^6–10^{10} times greater than signaling proteins that constitute biomarkers for disease detection—a sensitive cellular-level microfluidic device for proteomic analysis would therefore need to be able to detect proteins at such low concentrations,[163] or incorporate enrichment steps that involve preseparating out the proteins in abundance, for example, using solid phase extraction adsorption media or affinity-purified polyclonal antibody binders packed into on-chip liquid chromatography columns.[155,164]

Almost all workflows associated with protein identification using mass spectrometry require proteolytic steps, which typically involves the enzymatic digestion of proteins into peptides, either using in-solution, solid-phase, or in-gel approaches.[133] Protein digestion in-solution is usually slow and requires high temperatures, which could be problematic, and hence solid-phase digestion, which employs proteolytic enzymes immobilized or adsorbed onto the surface of a solid support, is often preferred. Solid-phase digestion can essentially be carried out in a microfluidic device by packing trypsin-derivatized beads into the microchannel[165] or by enzyme-immobilization onto solid monolithic supports.[166] Traditionally, in-gel digestion has not been popular in miniaturized proteomic analysis systems because of the inherent difficulty in incorporating the process onto a microfluidic chip as well as its lengthy sample processing time. Nevertheless, the possibility of reducing the entire in-gel digestion process, including sample preparation, rehydration, in-situ digestion, and peptide extraction from gel slices or spots, from several hours to under 30 min onto a microfluidic chip with the use of SAWs[21] has recently been shown.[167]

However, there has yet to be realized a fully integrated and automated high-throughput microfluidic proteomics analysis system that carries out the complete range of necessary functions from the introduction of single cells, for example, its lysis, and protein extraction, to sample handling, purification, preparation and separation, and subsequently delivery into a mass spectrometer through an appropriate interface for detection, all entirely on a single flow-through device with the capability of sequentially handling multiple streams (multiplexing).

3.4. Protein Crystallization

Protein crystallization is the most important step in X-ray crystallography, which is commonly employed in structural biology for the determination of 3D tertiary macromolecular structures and protein–ligand interactions. Achieving reliable and reproducible diffraction-quality crystallization is however inherently difficult due to the fragility of the crystal structure. Rigorous procedures are therefore required to ensure crystal purity and homogeneity; free interface diffusion, microbatch, vapor diffusion, and dialysis methods being commonly employed techniques. Nevertheless, the empirical nature of these processes typically necessitate trials involving the mixing of target proteins with a combination of precipitation agents and buffers, therefore rendering it a severe bottleneck

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in structure-guided drug design. Several microfluidic designs have been proposed as a way to address this rate-limitation and to preserve the strict quality control required in the crystallization process. Specifically, the ability for microfluidics to facilitate the handling of multiple samples, carry out parallel combinatorial reactions on a chip, enhance mixing in a precisely controlled manner, and provide an interface for evaluating the crystal quality makes it an ideal candidate for protein crystallization.

Higher crystallization rates are usually associated with free interface diffusion methods although the precise fluid manipulation required to achieve pure diffusive transport driven by concentration gradients has made this method challenging at conventional laboratory scales. Such handling and control of small fluid volumes is, however, quite routine using microfluidics. For example, a microfluidic device has been developed in which valves connecting separate microchambers containing the protein and precipitant solutions are opened to allow them to mix by diffusion.[168] Although precise, the use of pressure-activated mechanical valves can involve complex architectures and require large equipment ancillary to the chip device. A simpler device utilizing free interface diffusion is the SlipChip which simply requires slipping a top plate containing preloaded protein solutions in microchambers over a bottom plate containing preloaded precipitant solutions such that the microchambers are brought into alignment to allow diffusion to proceed (**Figure 12**).[169]

Instead of microchambers, droplet microfluidic systems[170,171] can also be used wherein the protein, buffer and precipitant solutions are allowed to form and mix within individual plugs separated by an immiscible fluid phase to constitute a combined microbatch and vapor diffusion platform for protein crystallization. The crystallized structures formed within the plugs downstream can then be transported for inspection using on-chip X-ray diffraction.[172]

In addition, microfluidic technology can also be harnessed to enhance the crystallization process. For example, the application of AC electric fields at frequencies commensurate with the characteristic protein hydrogen bond rotation time scale is believed to aid the desolvation of the hydration cages that surround the solvated protein molecules and shield the electrostatic interactions between them that are required for crystallization to occur. DEP can also be simultaneously employed to aggregate crystals in low field regions to form larger crystals.[20,173]

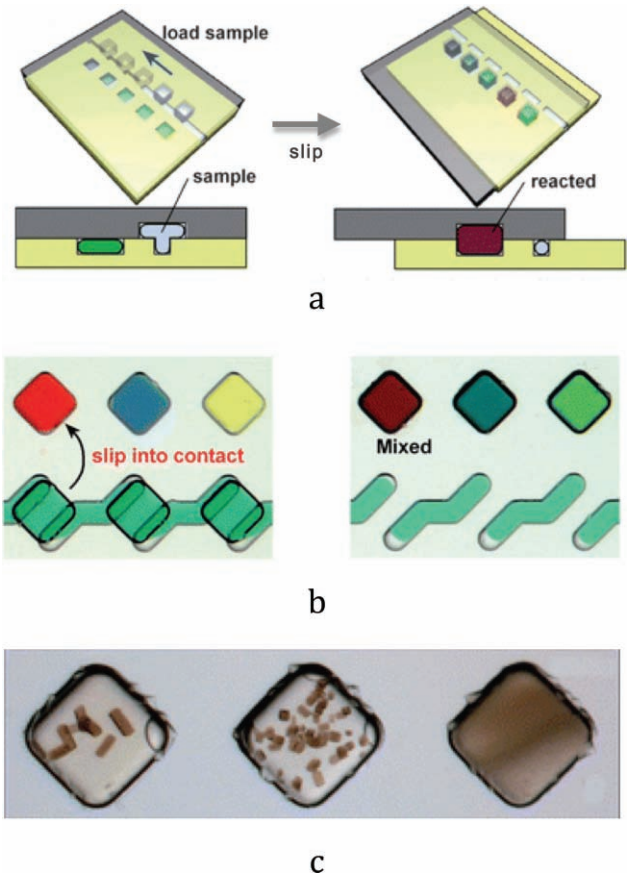

Figure 12. a) Schematics illustrating the operating principle of the SlipChip, in which separate rows of wells preloaded with precipitants and proteins on two different chip substrates are brought into contact by slipping the substrates over the other. The microphotographs below show b) the mixing of the model dyes, and c) the resulting protein crystals which form when the wells are brought into contact after slipping. Reproduced with permission.[169] Copyright 2009 The Royal Society of Chemistry.

4. Cellular Systems

Despite the long history of advances in molecular and cell biology, life science researchers still face considerable difficulty when trying to mimic typical in vivo cellular environments in order to increase the biological relevance in their study of human cells. This is because cells in their local environment constantly interact, either mechanically or biochemically, with other neighboring cells and the extracellular matrix. These spatiotemporally varying cues regulate the physiology, phenotype and fate of the cells and are hence an important consideration in cell culture and analysis. Microfluidics, however, offers the compelling opportunity to conduct these tests in an environment that can be carefully and precisely regulated in a controllable manner to replicate such cues on a local cellular resolution level, and promises to open a new dimension for understanding cell behavior and growth not possible through conventional laboratory-scale procedures.[3,174]

4.1. Cell Culture

In many ways, the scale down of bioreactors for cell culture is advantageous given the large surface-area-to-volume ratio associated with microfluidic systems. For example, microscale cell culture systems allow the incorporation of more efficient mass exchange networks to facilitate the perfusion of oxygen and nutrients to the cells, as well as the removal of waste (see also Section 9.2.3 on the incorporation of vasculature in scaffolds). Moreover, the small volumes involved necessitate only minute amounts of biological factors and produce less waste, which are particularly attractive for expensive bioassays or

cell culture studies. Polydimethylsiloxane (PDMS), which is highly permeable to gases, is a particularly attractive material for the fabrication of microfluidic cell culture platforms as it enables sufficient oxygenation of cells. Nishikawa et al.[175] demonstrated that a spheroid culture of rat hepatocytes can be stably attached to collagen-immobilized PDMS surfaces with an adequate oxygen supply that permeated through the bottom PDMS surface. Multiple layers of micromachined PDMS films can also be assembled into 3D microstructures for cell culture using oxygen plasma bonding.[176] In addition, most microfluidic bioreactors are optically transparent and compatible with conventional imaging techniques such that real time image analysis can be carried out. Cheap and disposable systems can also be fabricated to permit ease of assembly, cleaning and sterilization.

The ability to engineer surfaces, in addition, constitutes a flexible method for controlling interactions between cells, between the cell and the substrate, and between the cell and its surrounding medium (e.g., the extracellular matrix (ECM) or fluid suspension), both physically as well as biochemically, which are important factors that influence how multicellular organisms develop and function. For example, it has been shown that cell shape and morphology can be manipulated by way of cell–substrate or cell–ECM interactions introduced through topological surface modification in which alternate cell-adhesive and non-adhesive regions are patterned on a culture substrate.[177] This can be carried out by exploiting microfluidic channels to precisely control the delivery of proteins or polymers that act as cell-binding agents such they are adsorbed or immobilized along the microchannel.[178,179] It is then possible to selectively tune the adhesion and hence to pattern multiple cell types using different binding proteins to achieve the desired arrangement within the channel. It was also shown, for example, that the size of the fibronectin islands that the cells were patterned on and, therefore the cell adhesion density, had an effect on cell shape due to increased intercellular contact and paracrine signaling with increasing cell density—adhesion of cells to large islands yielded flattened cells that underwent osteogenesis during spreading to generate osteoblasts whereas adhesion of cells to small islands yielded spherical cells that underwent adipogenesis to produce adipocytes.[180] Varying the interstitial spacing between these regions has also been shown to regulate the cell spreading process through alterations of the cytoskeleton,[181] thus demonstrating the possibility for influencing cell fate such as controlling apoptosis or modifying the orientation of the cell division axis.[182]

Cell–cell and cell–ECM interactions arising from biochemical signaling or physical influences (e.g., mechanical force, temperature, or geometry) can also be controlled using microfluidic systems. Soluble signaling factors such as cytokines, hormones and growth factors secreted by cells diffuse and form chemical gradients, which are detected by neighboring cells, which then respond in the appropriate manner; neutrophils, for instance, migrate along soluble chemoattractant gradients released from an inflammatory site in a process known as chemotaxis. Both spatial and temporal gradients can play a role in chemotaxis. In the former, the concentration gradient of the signaling molecule across the cell is detected whereas the latter requires some memory of the concentration by the cell.

In recent years, microfluidic gradient generators have been developed via controlled diffusive species mixing within a microchannel network to manipulate both spatial and temporal regulation.[183] In a study of neutrophil chemotaxis under spatial interleukin-8 (a chemoattractant belonging to the cytokine family) gradients, Jeon et al.,[184] for example, fabricated a pyramidal branched network of microchannels to successively split, mix and recombine streams such that the flow through each microchannel sets up a different chemoattraction concentration with gradients perpendicular to the through flow direction that are gradient maintained over the channel length (see, for example, **Figure 13**). Temporal gradients, on the other hand, can be established through fast switching and reversal within a similar microchannel network with the aid of valves in which two important factors that influence the stimuli and response dynamics were reported—the average concentration over the cell and the average concentration at the leading edge of the polarized cell.[185]

The spatiotemporal manipulation of the local biochemical cellular environment afforded by microfluidic gradient generators has also been exploited to regulate the fate and function of stem cells. Using such a device to set up gradients in the growth factor concentration, it was reported that the proliferation of human neural stem cells was proportional to the growth factor concentration whereas the inverse was true for their differentiation.[188] Meanwhile, logarithmically varying concentration gradients and perfusion flow rates were established using a fluid resistance network connected to cell culture chambers to show that the growth of murine embryonic stem cells exhibited a strong dependence on the flow rate, due to the ability of the flow to deliver nutrients and to remove waste and secretion factors more effectively.[189] That cells respond functionally to mechanical stresses generated by fluid flow through changes in gene expression and cell morphology has been established—for example, the differentiation of skeletal myocytes are stimulated by tensile stresses, chondrocytes and osteocytes by compressive stresses, and, endothelial and muscle cells by pulsatile flow.[190] Such forcing, however, must be synergistic with chemical signaling pathways, as was shown for human embryonic stem cell differentiation.[191]

Long-term cell culture can be performed in microfluidic perfusion systems in which cells can be constantly fed with oxygen and nutrients, and metabolic waste products removed. An example of such a system employing a 2D 10×10 array of cell culture chambers from which the nutrient medium is perfused continuously from ports uniformly across the array is shown in **Figure 14**. The chamber arrays were also integrated with an upstream concentration gradient generator to enable cell assay studies to be performed with 10 different reagent concentrations in each array column, thus permitting the possibility of studying the effects of up to 100 different conditions.[192] A similar reporter array that allows the introduction of cells from the side ports through the array rows and the delivery of soluble factors for cell stimulation (inflammatory cytokines in this case) from top ports into the individually addressable cell chambers has also been

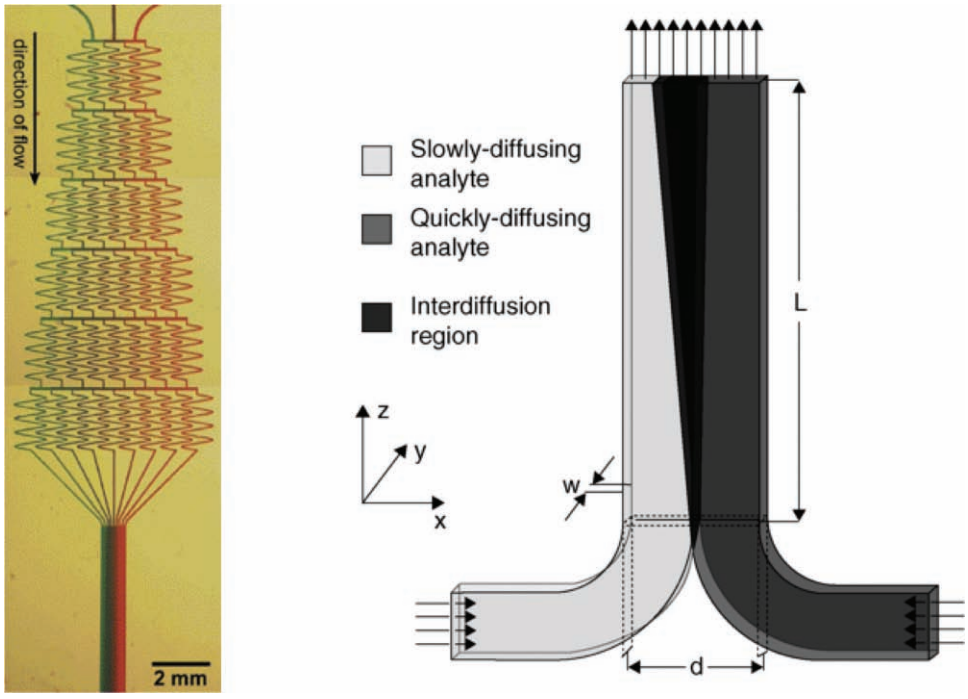

Figure 13. The left image shows a tunable microfluidic concentration gradient generator comprising a pyramidal branched network of microchannels, which are employed to successively split, mix, and recombine streams. The right image illustrates the gradient generated when two laminar streams are brought into contact such that they mix diffusively. Reproduced with permission. Copyright 2001 American Chemical Society.[186] Copyright 2008 Taylor & Francis.[187]

developed for the real-time monitoring of gene expression in live cells.[193] Although increasing the complexity of the device considerably, automation and control can provide the ability to optimize the seeding density, and medium composition and replenishment rate, such that it was possible to carry out unattended culture of human primary mesenchymal stem cells.[194]

Two-dimensional systems are however often poor replicas of the in vivo cellular microenvironment;[174,195] cells cultured in 2D systems have been known to lose their function or differentiation capability.[195] As such, there have been efforts to develop 3D cell culture systems, for example, the generation of patterned 3D microscale hierarchical tissue-like structure through sequential deposition of cells and biopolymer matrix on particular regions within microchannels.[196] Another example is through the fabrication of micropillar arrays within a microchannel on which cells are immobilized. In this case, the array is placed within the center of the channel along which a cell suspension is passed to deliver the cells; two side microchannels then flank the array to allow for flow perfusion.[197]

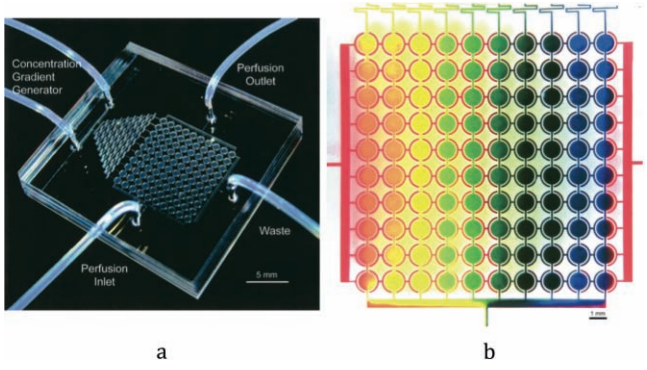

Figure 14. 10 × 10 Microfluidic perfusion array for cell culture with the capability for cell-based assays with multiple reagent concentrations (a). This is achieved by generating a concentration gradient, as illustrated in (b), in which red dye is perfused from the left port, and blue and yellow dye from two ports at the top, which also serve as inlet ports for loading the reagents and cells. Reproduced with permission.[192]

4.2. Cell Manipulation

Once cells are cultured and given the appropriate stimuli, the cells of interest need to be identified and separated for further analysis, requiring flow cytometry and sorting procedures, which, ideally should be integrated into the chip. After selection and separation of specific cells they are lysed, following which the lysate containing the membrane lipids, organelles, proteins, and nucleic acids need to be further separated to isolate the compound of interest for subsequent analysis.

4.2.1. Cell Sorting

Flow cytometry and cell sorting can essentially be carried out based on cell size, morphology, or, dielectric or magnetic properties. Conventionally, fluorescence-activated cell sorting (FACS) has been used by cell biologists. It can sort up to

10^4 cells per second although the systems available are usually large, cumbersome, and expensive, and the viability of a significant number of cells can often be compromised; miniaturized FACS sorters integrated onto microfluidic chips, on the other hand, can handle one order of magnitude of cells per second less and are restricted due to the small microliter volumes they are able to handle, but retain greater cell viability numbers.[198,199]

Microfluidic particle manipulation schemes, nevertheless, offer the potential for more precise particle maneuverability, increased sorting speeds and ease-of-integration. Laser-induced optical trapping, which imparts a pico-Newton order force on a dielectric particle due to the momentum transfer arising from incident photon scatter,[200] can provide an efficient means for moving and separating single cells in a microfluidic device[201] based on their morphology, size, and refractive index but requires the cells to be aligned in single file row as they flow toward the laser beam, which is a severe drawback for high-throughput analysis. While this can be circumvented by aligning the cells by hydrodynamic focusing,[202] the necessity of a laser and microscope in 'optical tweezer' techniques can not only be prohibitively slow and costly, but also restrictive to on-chip integration.

Magnetic fields can also be employed for isolating paramagnetic cells which exhibit magnetic susceptibility (e.g., erythrocytes)[203] or whose surface has been attached with magnetic nanoparticles.[204] Alternatively, bacterial cells, for example, can be selectively bound to larger magnetic beads with conjugated capture antibodies on its surface.[205] The low power requirement of magnetic sorting is attractive from the point of view of miniaturization and portability. In addition, loss of positioning accuracy due to Brownian diffusion can be eliminated with the use of magnets. Most work to date, however, has involved the use of external permanent magnets and electromagnets,[206] although there have been efforts to fabricate miniature magnetic components that can be integrated into the microfluidic device.[205,207]

Acoustics can, on the other hand, facilitate integrated on-chip particle manipulation. In Sections 2.1 and 2.2, SAW-driven microcentrifugal convection has been shown as a tool for preconcentration to facilitate detection at lower sensitivity limits, or, for example, to separate red blood cells from plasma (Figure 5).[45–47] This mechanism can also be used to separate two different particle species based on size.[208] SAWs, when induced as standing waves, can also constitute a rapid 'acoustic tweezer' mechanism for cell alignment, patterning and positioning, using significantly lower power than that required with optical tweezers, and can be employed together with flow cytometry.[209]

While acoustic fields comprise an unsurpassed force to manipulate particles in terms of speed, the sound wavelengths associated with currently accessible excitation frequencies (typically 10–100 MHz with SAW and lower with bulk ultrasound) are too long for single cell manipulation at present. Electrokinetics, however, offers such capabilities for precisely manipulating single cells and biomolecules.[20,210] Electrophoretic separation has been widely used with bacterial and eukaryotic cells and even subcellular organelles.[211] In the latter, the protein content from the organelles obtained from sonically homogenized epithelial esophageal cells were digested and separated using 2D capillary electrophoresis, which combines two electrophoretic techniques, in this case, capillary sieving electrophoresis and micellar electrokinetic chromatography, to increase the separation resolution by co-axially mounting two aligned capillaries in an interface.[212] Nevertheless, electrophoretic separations necessitate large DC voltages, which not only prohibit miniaturization and integration due to the cumbersome power supplies required, but are also prone to bubble generation which often suppresses the electro-osmotic flow within the capillary.[20]

DEP, which employs a non-uniform AC electric field to induce interfacial polarization and hence an effective dipole on a particle suspended in a medium, is a powerful tool for particle manipulation and sorting and can circumvent the problems associated with the use of DC fields in electrophoresis (see also Sections 2.2 and 2.3.2).[20] It should be noted though that a particular cell's behavior under DEP can be altered if there are changes to the physical composition of its membrane.[213,214] While this means that one should be aware that prolonged storage or sample handling, which could potentially alter the cell's membrane characteristics, can affect sorting procedures,[215] such dependence could also provide opportunities for sorting the same type of cells merely based on their membrane characteristics, for example, the cell age.

DEP is, nevertheless, slow (approximately 10 μm s^{-1} for bacterial cells, for example) and short range as the DEP force scales quadratically with the applied voltage (which is often practically limited) and as the cube of the particle size. It therefore cannot provide a means for high-throughput particle sorting as the continuous-flow throughput is below 25 nL s^{-1} with the 10 μm s^{-1} DEP velocities achieved.[215] Long-range hydrodynamics can however provide a solution to this problem by convecting particles to a local stagnation region where DEP forces can be imparted to finely manipulate the particles to the desired sorting order.[20] There are many ways that long-range convection can be bestowed on the bulk particle suspension; some of these have been discussed previously in Sections 2.1 and 2.2 in the context of sample preconcentration or blood plasma separation, viz., acoustic[45–47] or electrohydrodynamic[40–44] microcentrifugation. Alternatively, AC electro-osmotic flow can also be employed.[216,217] A fast continuous-flow integrated DEP chip that employs 3D electrodes to successively filter debris, focus and sort bacterial cells and finally trap them within bins without the necessity of external pumps or ancillary laboratory-scale infrastructure is also shown in Figure 2.[32]

4.2.2. Cell Lysis

Methods for carrying out cell lysis have to be incorporated into integrated cell analysis devices. There are a number of ways that cells can be lysed by rupturing its membrane, all of which can be easily integrated into a microfluidic device. Chemical lysis involves the use of enzymes (lysozymes) or non-ionic detergents and has the advantage of easy-integration by dispensing the chemical from reservoirs; moreover,

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subsequent assays can be carried out within the same buffer solution.[174]

Other lysis methods are possible if the chemicals pose a problem to subsequent analysis. Thermal effects similar to the first stage in PCR (see Section 2.4) can be applied to lyse cells but could potentially denature the proteins in the lysate.[218] Mechanical lysis, on the other hand, can be induced through the application of shear, brought about, for example, by fabricating nanoscale knife-like ridges within a filtering region that the cells are made to pass.[218] Nevertheless, the necessity for such complex fabrication procedures render the device difficult and expensive to manufacture. In addition, despite the efficiency of lysing the cells, the resultant cell debris mixed with the lysate resulted in low recovery efficiency of the proteins for further analysis.

Alternatively, sonication can also be employed although this requires the use of large ultrasonic horn transducers[219] not amenable to miniaturization. Sufficiently high-intensity electrical pulses can also cause cell poration by inducing transient and localized instabilities along the cell membrane. Indeed, electroporation (also known as electropermeabilization) has been used to introduce nucleic acids into cells for gene transfection simply by fabricating electrode arrays in a microfluidic device[220,221] (see also Section 8.3 on gene delivery), and incorporated into integrated chips for cell culture and analysis.[222] Such ease of integration has also rendered electrically induced cell lysis a commonly used technique.[223,224]

4.3. Cell Analysis

Despite significant advances, microfluidic cell analysis remains challenging and has, to date, been limited to DNA/RNA analysis or the analysis of less complex samples that do not require complicated fractionation and processing steps; there is still much research and development that has to be carried out before real biological samples, which involve protein extraction and analysis (Section 3) from whole cell lysates or blood plasma, can be accurately and reproducibly handled.[174] The majority of cell analysis has been conducted through a combination of off-chip and chip-based procedures, since multiplex microfluidic devices which integrate the entire extent of processing steps from cell culture, treatment separation, and analysis have only been developed recently. As microfluidic genomic and proteomic analysis has been covered in Sections 2 and 3, we will restrict our discussion here to cell-based biosensor systems and single-cell analysis; other cell analysis systems such as cell microarrays and chemotaxis assays have already been briefly touched upon in Section 4.1.

4.3.1. Cell-Based Biosensors

The physiological responses of cells to stimuli released by biological agents can be exploited for pathogen or toxin detection,[225] drug testing,[226] or environmental monitoring.[227] In combination with the precise control and regulation of microfluidic cell handling and manipulation, such monitoring of cell electrophysiological activity using microelectrodes, intracellular signaling using optical probes (e.g., fluorescent dye probes or reporter genes), variations in cell morphology using electrical impedance spectroscopy, changes in cell metabolism using thermometric calorimeters, or even the distinction between live and dead cells through changes in the membrane electrical resistance, has given rise to the possibility of portable cell-based biosensors.

There are, however, a few challenges that have so far limited the use of cells as biosensors. By far, the greatest difficulty posed is the inability to reliably reproduce the cells' response due to the large biological variability associated with living cells, including cell–cell and cell–matrix variation.[174] The sensitivity of the cell to a wide range of chemical stimuli also makes it hard to achieve the specificity required in functional biosensors. Integration of sample preparation steps, cell regulation procedures as well as electronic data monitoring circuits are also necessary. A PDMS cartridge that houses the cell and a complementary metal oxide semiconductor (CMOS) silicon chip, which provides a digital interface that incorporates temperature control, electrophysiological sensing and analog signal buffering, has been developed as a biosensor to monitor cardiomyocyte signaling in response to the introduction of biochemical stimulants.[228] Cardiomyocytes are a good choice of electrically active cells for electrophysiological sensing as they form a connected network of spontaneously beating syncytia with large coverage area, which is a requirement for good detection of extracellular action potential signals. Another widely used cell candidate is the neuronal cell. While their coverage is more sparse and less confluent, this is compensated by the axons that asynchronously conduct electrical impulses away from the cell soma. Moreover, the possibility of patterning an assembly of neuronal cells in a network along electrode arrays has been demonstrated;[229] the connectivity of these synaptic networks can also be confined using microfluidics to physically guide their growth along topologically patterned substrates.[230]

4.3.2. Single-Cell Analysis

With the advent of microfluidic single-cell assays, cell biologists now have the opportunity to investigate cells on an individual basis, given that variability, for example, in the expression of a specific gene, exists even in identically-apparent cells and cannot be captured in aggregated data averaged over the large cell population afforded by traditional assays. Yet, such variability information on a cell-by-cell basis could lead to a better understanding of disease towards the goal of personalized treatment. In cancer, for example, the proteomes in similar cells progressively differ at an increasing rate. While microfluidic devices have dimensions quite appropriate for single-cell analysis, technology has still to progress to overcome the challenges with proteomics outlined in Section 3, for example, the large dynamic range in protein concentration in a cell.

The complexity of the cellular components also requires the integration of the full range of sample preparation and purification procedures described previously. There has been

some progress with regards to such integration. For example, single red blood cells have been loaded, docked, and lysed using electric fields, then electrophoretically separated for subsequent detection of an intracellular component glutathione using laser-induced fluorescence.[231] Single NIH/3T3[94] and T-lymphocyte[232] cells have also been captured and lysed, followed by reverse transcription of isolated mRNA into cDNA and subsequent amplification via PCR (RT-PCR), fragments of which are captured via an affinity matrix, and electrophoretically separated for gene expression profiling; in addition, it is possible to incorporate multiplexed PCR amplification to facilitate higher throughput[233] or to interface the electrophoretic separation with ESI-MS[234] (see Section 3.2.2).

Another difficulty with single-cell analysis relates to the ability to analyze the contents of a single lysed cell since they diffuse out of the cell into the buffer and are hence rapidly dispersed, thus resulting in significant band broadening.[235] This problem can however be overcome by encapsulating individual cells into droplets immiscible with the buffer solution so that the dilution of the cellular contents and their subsequent dispersion is minimized. High-throughput procedures utilizing droplet microfluidic platforms[170,171] for the screening of single-cell viability and growth or the measurement of reporter gene expression have been reported, as exemplified in the illustration in **Figure 15**.[236]

Single-cell analysis can also be applied to the investigation of ion channel activity, which has important consequences for drug screening. Two methods are generally employed for chip-based ion channel monitoring: patch clamping and fluorescence assays. Patch clamping is an electrophysiological approach which can provide detailed current information through the ion channel but is slow, laborious, and costly to use. Chip-based systems, which, for example, employ fabricated 3D micronozzles integrated into a microfluidic device,[238] however, can increase throughput with reduced costs. Another integrated patch-clamp setup traps the cells in lateral microchannels and employs a negative pressure to draw the cell towards the patch channel in a similar manner to lateral patch-clamp designs.[239] Fluorescence assays, on the other hand, can facilitate high-throughput usage but conventionally require large numbers of cells and have poor signal-to-noise ratios. With the use of two membrane-permeable anionic and cationic fluorophores whose rate of uptake in the cell is dependent on the membrane potential, it was shown that costs as well as the number of cells per sample could be reduced considerably by carrying out the assay in an automated microfluidic device.[240]

5. Biosensors for Biochemical and Pathogen Detection

Various miniaturized versions of biosensors, which is the broad term given to analyte detection associated with biological compounds, as they relate to genomic, proteomic, and

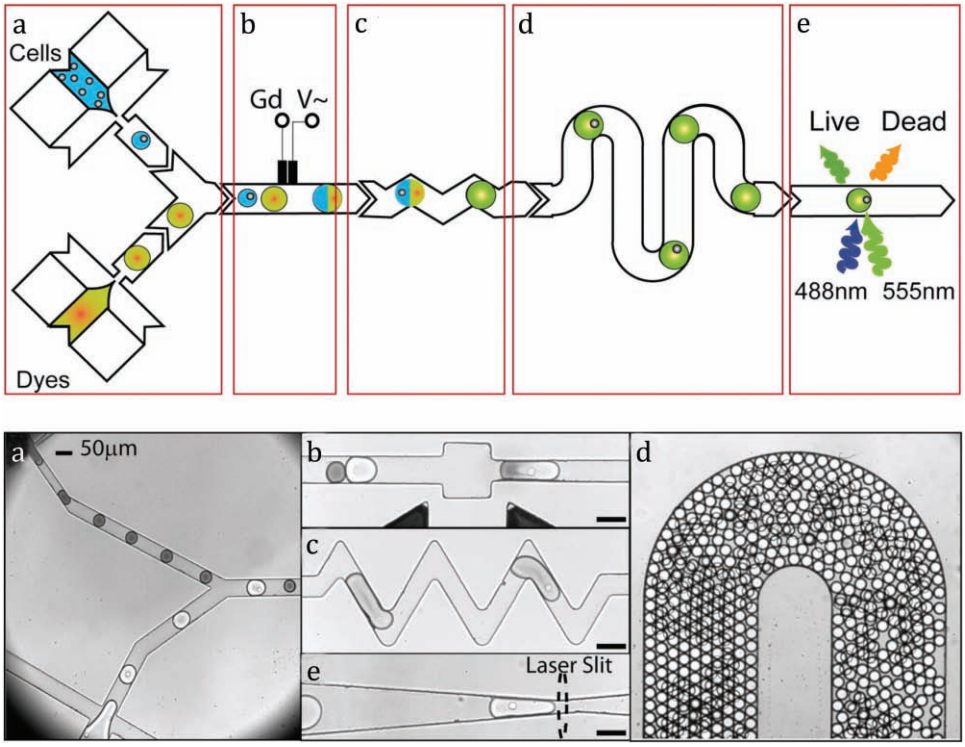

Figure 15. Cell viability assay based on a droplet microfluidic platform. a) Cells and fluorescent dyes are encapsulated in the droplets, which are then introduced into a merged channel in such a way that they alternate in sequence. b) Using an AC electric field, the adjacent droplets containing the cell and the dye are merged. c) Mixing within the droplets is induced by a series of sawtooth channel patterns. d) The droplet then passes through serpentine channels to increase the residence time to allow for sufficient incubation over 15 min. e) The droplets then pass through a detection region where live and dead cells can be sorted. Reproduced with permission.[237] Copyright 2009 National Academy of Sciences, USA.

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cellomic analysis, have been discussed in the preceding sections. In this section, we further elaborate on two themes, viz., chip-based biosensors for enzymatic and pathogen detection, due to their relevance to a wide range of biomedical, environmental, food, and chemical applications.

5.1. Microfluidic Enzymatic Assays

Besides nucleic acid and protein approaches, and immunoassays, enzymatic assays for synthesis, chemical modification, and cleavage are commonly used for clinical diagnostics; other uses of enzyme reactions are organic synthesis, metabolite waste removal, blood detoxification, peptide mapping, and detection of post-translational modifications.[241] One example that is in widespread use is the ubiquitous enzyme strip for the estimation of blood glucose, cholesterol, and electrolyte levels. Accurate and reproducible enzymatic assays are, however, difficult to scale down in size because of the strong dependence of assay performance on system parameters such as the enzyme concentration, applied voltage, and microchannel dimensions.[242] Nevertheless, the benefits of miniaturization, which include the possibility of portable food and medical diagnostic kits as well as lower costs and faster analysis due to the reduction in sample volume, has spurred activity in the development of chip-scale enzymatic reactions in recent years for analyte species quantification, reaction kinetics evaluation, and inhibitor assessment.

Enzymatic assays are either homogeneous, in which the reactants are held in the same phase, i.e., in solution, or heterogeneous, in which the enzyme is immobilized on the surface of the device or on that of a solid support. Heterogeneous reactions are attractive because they allow ease of enzyme loading and recycling.[243] Early microfluidic devices for conducting on-chip enzymatic reactions were homogeneous. One example is a device to evaluate enzyme kinetic parameters as well as to assess effector (inhibitor or cofactor) performance in which the reactants (enzyme, substrate, and inhibitor) were electrokinetically metered into a reaction chamber to control the dilution and mixing, and the reaction kinetics monitored via laser-induced fluorescence.[244] In order to circumvent the necessity for off-chip optical fluorescence detection, Kang and Park[245] developed a miniature enzyme assay platform that adopts the dilution capability of a microfluidic gradient generation device (see Section 4.1) in a parallel channel network format so that it fits a microtiter plate reader. Simultaneous measurement of sequential dual-enzyme reactions has also been performed,[242] for example, for glucose-lactate monitoring, combined with capillary electrophoresis to separate the substrate and reaction products and amperometric detection of the hydrogen peroxide product.[246] Heterogenous enzymatic reactions for continuous-flow systems, on the other hand, have been carried out by immobilizing the enzymes onto the microchannel surface or onto microbead supports over which the substrate solution is passed.[241,243]

Integrated and automated devices for simultaneous enzymatic assays have also been recently developed. One such device for the investigation of enzyme kinetics allows the simultaneous reaction of eleven reactions in parallel, and integrates sample metering, mixing, and incubation capabilities.[247] Another device, which facilitates the quantification of hydrogen peroxide for monitoring oxidative cellular damage, integrates sample transport, mixing and dilution, and rinsing using an automated 2D valve actuation platform.[248] Herr et al.,[249] on the other hand, developed a diagnostic device that integrates sample filtering, enrichment, and mixing to carry out an enzymatic immunoassay that measures the levels of a tissue-decaying enzyme in human saliva as a means for quantifying the extent of periodontal disease. Their technique, which employed electrophoretic separation through molecular sieving gels of the enzyme bound with a fluorescently labeled monoclonal antibody complex with the native antibody and subsequently detected using laser-induced fluorescence, negated the need for antibody pair matching and capture antibody immobilization. In other work, single-cell enzyme kinetics has also been studied in a large cell trapping array integrated with sample preparation procedures.[250]

5.2. Microfluidic Pathogen Detection

One of the more unfortunate ramifications of the era of unprecedented cheap and rapid global air travel is the rapid promulgation of infectious diseases, evidenced by recent pandemic risks and outbreaks. The recent growth in terrorism activity has, in addition, led to increased risk of biological warfare. The demands for rapid screening of airborne, waterborne, and foodborne pathogens and biological warfare agents placed by these factors render conventional methods for detecting and identifying micro-organisms ineffective, as they are often tedious, cumbersome, time consuming, and costly. With microfluidic technology, pathogen detection can be conducted much faster, more accurately, and with greater sensitivity in a portable device.

Detection can be carried out in a variety of manners, all of which can be coupled to a miniaturized device.[251] Among the most popular detection schemes are electrochemical (e.g., impedance spectroscopy, amperometry, potentiometry) and optical (e.g., fluorophore conjugation of affinity markers, chemiluminescence, bioluminescence, surface plasmon resonance) methods, although other techniques such as acoustic, mechanical, PCR or RT-PCR, mass spectrometry, nuclear magnetic resonance, or magnetoresistive sensing have also been widely used. The necessity for large ancillary detection equipment external to the microdevice, however, remains the biggest limitation to true on-chip functionality. In general, microfluidic pathogen sensing can be subdivided into three broad categories, viz., through the detection of DNA/RNA sequences or proteins, quantification of metabolites consumed or released due to pathogenic activity via enzyme-based detection, and by carrying out whole cell-based assays,[215] all of which have been covered in the preceding sections. The necessity for multipathogen detection in practical biosensors, however, imposes the need for microfluidic devices that can simultaneously process multiple samples without cross-contamination whichever detection pathway is adopted.

Another fundamental limitation to rapid and efficient pathogen detection is the trace quantities of microbes typically

present in samples, even though such low concentrations are often potentially lethal. Compounding this problem is the large number of other species, for example, blood cells, in the sample. In addition, the transfer of 'real-world' milliliter samples to nanoliter microfluidic devices necessitates sample preconcentration steps; this is however often associated with large sacrifices in detection sensitivity. Although advanced detection schemes of growing sophistication to carry out detection with increasingly higher sensitivities continue to be developed (see, for example, the acoustically and electrohydrodynamically driven microcentrifugation techniques[41–47] discussed in Sections 2.1 and 2.2, in which sample preconcentration is accompanied by an amplification in the detection signal), albeit at the expense of increasing complexity or higher costs, microfluidic sample preparation procedures for target isolation presents a cheaper alternative that can not only increase target specificity but also the concentration of the target simultaneously. Some microfluidic techniques for target isolation such as cell culture (Section 4.1) and magnetic or electrokinetic separation (Section 4.2.1) have been discussed previously.

Microfabricated filters[252] (e.g., hole or pillar arrays) or the use of porous matrices incorporated into microchannels are also another simple way to separate target molecules and cells although they could be complex and expensive to fabricate, and are often associated with large pressure drops or prone to fouling, thereby necessitating the use of large capillary pumps that are not amenable to miniaturization. The large aspect ratio of nanowires and nanotubes can also be exploited for target isolation and transport due to their enhanced conductivity and induced dipole field, which serve to attract biomolecules as well as to amplify DEP velocities.[36,253–255]

6. Drug Development

Screening for potential drug candidates is a costly affair, typically taking, on average, around 13 years and costing over approximately US$800 million from the inception of a drug candidate until it appears on the shelf. Only a mere fraction, around 10%, of drug candidates make it all the way to market, and, more alarmingly, almost half of the drugs fail at Phase III clinical trials where 80% of the costs have already been incurred.[256] Microfluidics offers a potential solution for reducing the time and costs required at every stage in the drug discovery workflow, from automated genetic and proteomic handling and analysis platforms (Sections 2 and 3) in the target identification stage, combinatorial format microreactors for compound synthesis, cell sorting, and manipulation techniques (Section 4.2) or protein crystallization platforms in the lead identification stage for structure-guided screening (Section 3.4), and, cell (Section 4) and tissue (Section 9) analysis systems in the lead optimization stage for drug efficacy and toxicity studies and pharmacological profiling.[5]

6.1. Enhancing Chemical Reactions

Microfluidics can enhance on-chip chemical synthesis in a number of ways. The enhanced surface-area-to-volume ratio in microscale devices can be exploited to increase access of reactants over shorter diffusion length scales to an increased catalytic surface area, which could either be patterned over the microchannel surface, embedded into monolithic supports held in place in a microchannel, or dispersed as beads in the flow system. Given the absence of turbulence in low-Reynolds-number microfluidic systems and since kinetic time scales are relatively short, microscale reactions are typically diffusion-limited; introducing micromixing, either passively by introducing flow lamination[257] or fabricating flow obstruction structures,[258] or actively through the judicious application of external forces (e.g., electrokinetically or acoustically, for example, to generate microcentrifugal vortices[40,42,44,47,259]) to reduce diffusion length scales by breaking up the laminarity of the flow or to induce chaotic convection, is another way in which reaction times can be reduced considerably.[260]

Reactions at the microscale can also be accelerated, for example, by using the efficient SAW microfluidic energy transfer mechanism between the substrate and the fluid[21] as an energy source to overcome activation barriers while exploiting the large surface area per unit volume typical in microfluidic systems for enhanced heat transfer. With merely 1 W input power, one or two orders of magnitude lower than that used typically in sonochemistry or microwave-assisted chemistry, it was demonstrated that a range of normally difficult organic reactions requiring high temperatures and pressures over long periods of time (hours or days) can be carried out much faster (seconds or minutes) with comparable or even higher reaction yields.[261]

Microfluidic combinatorial operations also offer the synergistic opportunity of both miniaturization and parallelization to achieve rapid synthesis outcomes without requiring large volumes of expensive reagents.[262] A 3D 2 × 2 combinatorial library microreactor for amide synthesis using phase transfer[263] is shown in **Figure 16**b (compared to a conventional combinatorial system which carries out the synthesis in parallel using four individual microfluidic chips as shown in Figure 16a) though a higher order $n \times m$ system increases in complexity considerably given that a 3D network is necessary to capture all possible combinations in the mixing of $n + m$ reagents.[5]

6.2. Enabling High Throughput

Screening library compounds in the lead identification stage against drug targets is a formidable task given the enormous number of possible lead compounds. This is conventionally carried out either through solution-based biochemical assays (e.g., enzyme inhibition or receptor-ligand binding; see, for example, Section 5.1) or cell-based assays (e.g., reporter gene assays to evaluate transcription/translation level cellular response, cell proliferation assays to monitor cellular response to external stimuli, or second messenger assays for monitoring cell–surface interactions via signal transduction measurements; see, for example, Section 4).[264] By miniaturizing the size of each miniature solution well used in homogeneous biochemical assays, a larger number of wells can

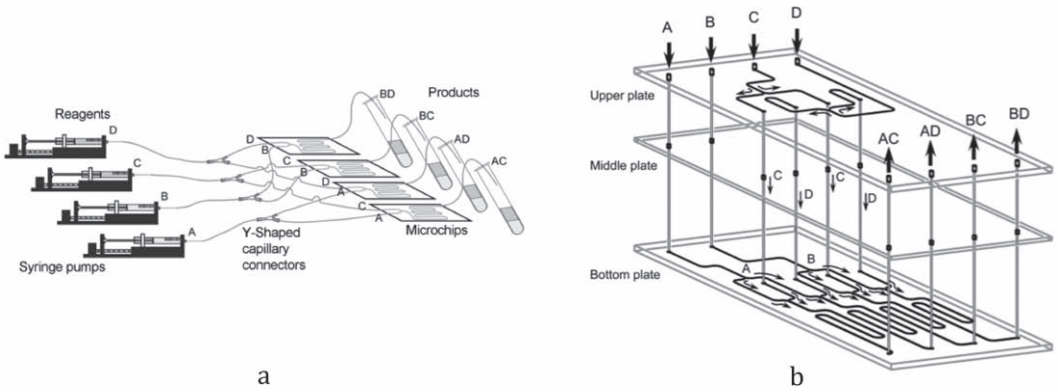

Figure 16. Schematic illustration of a) a conventional parallel microreactor system comprising four parallel microfluidic devices, and b) a 3D microreactor chip to carry out a 2×2 combinatorial amide synthesis. Reproduced with permission.[263] Copyright 2002 The Royal Society of Chemistry.

be incorporated into a microarray biochip. With the higher density 1536-well microarrays, high-throughput screening can now screen over 100 000 compounds a day. Nevertheless, the current well sizes are close to their minimum dimension limitations. Moreover, batch screening of individual microarray well plates is inefficient due to the limitations in the speed and precision at which small amounts of fluid can be dispensed by robotic micropipetting systems; with these small well volumes, sample evaporation also poses a considerable concern.

Continuous-flow microfluidic chips with integrated on-chip components for sample handling, transport, mixing, reaction, separation, and screening provide a more efficient way to screen potential drug candidates over batch microarray testing and are more convenient to be transferred for storage and testing. Moreover, with closed systems, problems with evaporation and reagent exposure to the atmosphere are suppressed. One example that demonstrates the potential use of microfluidic concentration-dependent binding assays for lead identification is a microfluidic titration chip to study the interference effect of the anticancer drug doxorubicin on the binding of the transcription factor Sp1 to DNA. The chip comprised a concentration gradient generation network (see Section 4.1), each arm of which serially integrates a mixing serpentine channel, a fluorescence correlation spectroscopy interrogation chamber and a waste outlet.[265]

Other continuous-flow devices, for instance, adopt droplet microfluidic systems[170,171] in which individual reagents are compartmentalized within nanoliter plugs in an immiscible liquid carrier medium and transported within a microchannel as shown in **Figure 17**a. At a T-junction, the substrate solution is then dispensed into the reagent plug. This concept was also extended to carry out enzymatic functional assays. In this case, the plugs consist of enzyme solutions with phosphatase activity that fluoresce when the substrate is introduced into the enzyme plug at the junction (Figure 17b). In this case, plugs of buffer solution were placed adjacent to each enzyme plug to eliminate cross-contamination; to prevent coalescence between the plugs, gas bubbles were formed between neighboring enzyme and buffer plugs.[266]

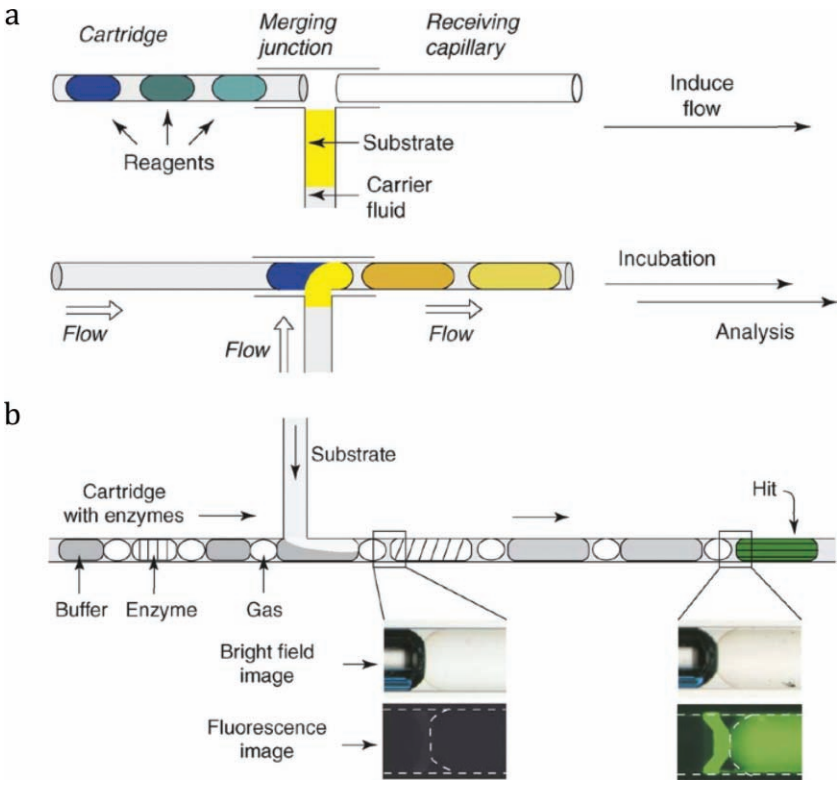

Figure 17. Droplet microfluidic platform for a) compartmentalizing and transporting reagents. The substrate solution is dispensed into the droplet containing the reagent at the T-junction. A similar procedure can be employed to carry out b) enzymatic functional assays. The phosphatase activity fluoresces when the substrate is introduced into the droplet containing the enzyme at the T-junction. Droplets containing the buffer solution are introduced between the droplets containing the enzyme to minimize cross-contaimination whereas gas bubbles were introduced between these droplets to prevent coalescence. Reprinted with permission.[266] Copyright 2006 Elsevier.

Integrated microfluidic-based arrays for cell-based approaches have also been developed, some of which have been reviewed in Section 4. The multiplexed microfluidic system fabricated using multilayer soft lithography comprising a network of microfabricated valves and individually addressable wells shown in Figure 8 has been used to screen *E. coli* cells for green fluorescent protein expression.[92] Similar live cell transcriptional reporter monitoring has been conducted in a 2D cell microarray with integrated microfluidic gene and transcription factor seeding and perfusion culture system that allows each matrix element to be individually addressed and monitored after application of molecular soluble factors.[193] A high-throughput device to conduct cytotoxicity assays, which is a mainstay method for drug screening, based on a platform similar to that in Figure 8 has been developed to analyze the exposure of three different cell types to five different toxins.[267] Both biochemical and cell assays have also been combined in a high-throughput microfluidic platform that screens several antithrombotic agent candidates against human platelet receptor targets by ratiometric measurement of the calcium flux released upon agonist stimulation of the platelet cells.[268] In the examples above, the ability to rapidly and efficiently confine single cells or reagents in a separate compartment, whether in a well, trap, or droplet, so that cross-contamination is eliminated, and the possibility for treating individual cells or reagent volumes sequentially and in parallel has made microfluidic platforms extremely appealing for high-throughput drug screening.

7. Point-Of-Care Diagnostics

Long patient wait times and limited access to diagnosis and treatment are now common in many countries as a result of years of chronic underfunding of healthcare systems. Bringing diagnostics to the point of need could potentially allow some form of preliminary self-screening or sorting and even basic treatment by front-line nursing staff, therefore reducing the burden on practitioners and hospitals and allowing specialist care to be dedicated to those most in need. Developed nations with established healthcare systems, however, are not the only potential beneficiaries of advances in point-of-care diagnostics. Cheap, portable, and easy-to-use diagnostic tests can also be employed to supplement the basic medical equipment available in third-world nations[4] or that used by healthcare workers or aid agencies for disease testing or in disaster relief. In addition, diagnostic test kits for field use are also indispensable in military, forensic, and space operations.

For technology such as microfluidics to be effective as point-of-care diagnostic platforms,[269] however, certain design criteria must be fulfilled. Devices need to be low-cost and portable, preferably handheld and disposable (single-use).[270] The entire platform must be simple, by way of user input and without requiring large and complex equipment, and ideally integrated in its entirety onto a chip. The assays must be fast, on the order of seconds and minutes, accurate, reliable, and reproducible, while being unambiguous and easily used and interpreted by the layperson without requiring training or regular maintenance. In military, space, and developing-world applications, these criteria are even more stringent. The device must be sufficiently robust to withstand operation in extreme conditions such as dust, wind, and pollutants, and must remain stable during transportation. It must be lightweight, especially in space applications, and self-contained; that is, it must have its own miniature power supply (e.g., battery operation) and must not require refrigerated storage conditions, especially when used in rural conditions where power is not always available. The complexity of its use and the interpretation of the result must be commensurate with the level of education in rural settings. More so in developed nations than in developing nations, however, where mail-order genetic testing and off-the-shelf home DNA test kits[271] are now widely available with HIV screening kits soon to follow, other nontechnical considerations also need to be given appropriate attention. For example, legal and ethical issues surrounding point-of-care testing, such as information privacy and theft concerns; the potential for abuse through testing that is either unsolicited or without specific informed consent; the implications of wrong, negative, or even positive test results without adequate pre- or post-test counseling; etc.

While early microfluidic devices were constructed out of silicon, building upon prior semiconductor microfabrication know-how using simple etching and photolithography procedures, subsequent advances in soft lithography[272] has opened up the possibility for surface patterning and device fabrication using cheaper and less fragile elastomeric materials such as PDMS,[273] thus facilitating the era of the disposable chip that can be easily interfaced with other devices through flexible fluid connectors. In addition, soft lithography is fast, low-cost and has the capability of multilayer fabrication to build up on-chip 3D structures. The high gas permeability and optical transparency of PDMS also allows enables easy on-chip cell culture and detection, respectively. One drawback of PDMS, however, is its incompatibility with organic solvents. Other fabrication techniques such as hot embossing and injection molding of thermoplastics such as polymethylmethacrylate (PMMA), polystyrene, and polycarbonates has also widened the versatility in the fabrication of disposable chips.

Paper-based microfluidic systems also offer a cheap and disposable alternative for simple point-of-care diagnostic applications. With origins dating back to the invention of paper chromatography and paper test strips in the early 20th century, which has given rise to simple pH and immunochromatographic testing, a well-known example of the latter being the home pregnancy test kit, more recent state-of-the-art paper-based methods include on-paper colorimetric sensing in which detection is based on color changes arising due to the aggregation of gold nanoparticles embedded into paper induced by biological analytes,[274] as well as the patterning of virtual microchannels and zones by modifying the wetting properties of paper (**Figure 18**); such patterning was employed for glucose and protein analysis in urine samples without requiring a power source (Section 3.2.3).[153,154] Overcoming the slow capillary-driven transport through paper and providing a means for extracting analytes from paper has been demonstrated using SAWs powered by two camera

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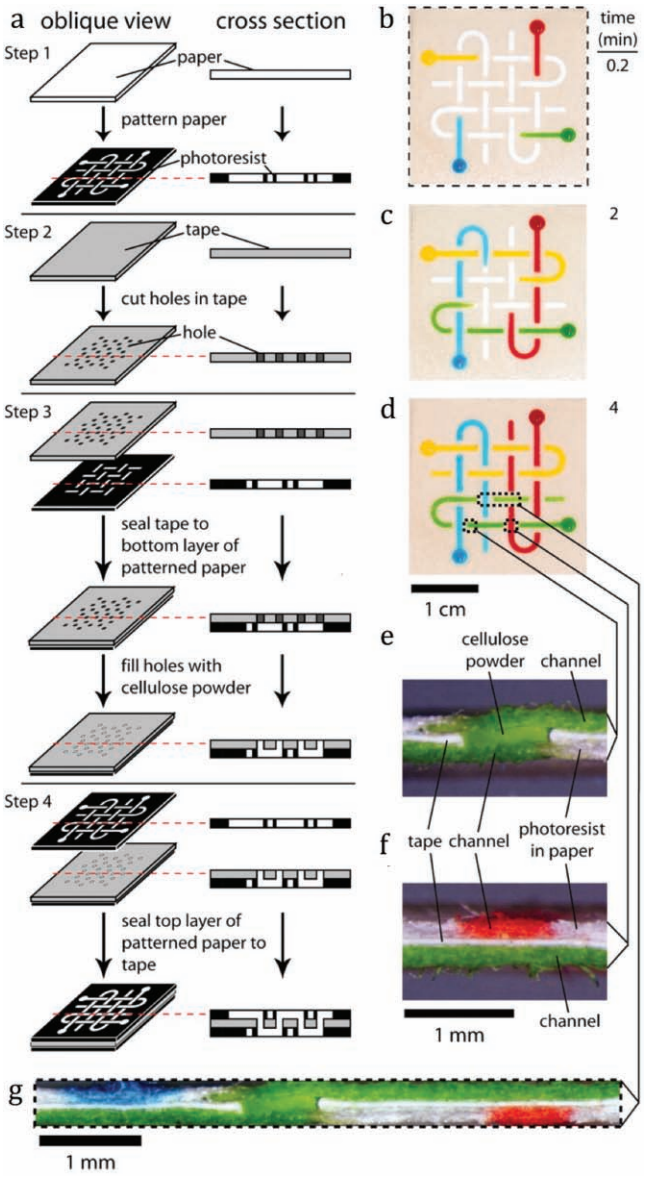

Figure 18. a) Schematic illustration showing a 3D microfluidics device for point-of-care diagnostics constructed simply by layering alternate sheets of adhesive tape and paper on which hydrophobic polymers have been patterned to form virtual channels, as shown in panels (b), (c), and (d), at times 0.2, 2, and 4 min after addition of the colored dyes, respectively. Cross-sectional images of the device in image (d) showing e) the top and bottom paper layers, f) three paper layers with channels orthogonal to the top and bottom layers, and g) the distribution of fluid in each layer. Reproduced with permission.[153] Copyright 2008 National Academy of Sciences, USA.

batteries.[122] While possessing the advantages of simplicity, biodegradability, as well as low operating and manufacturing costs, and, at the same time, combining more advanced fluid and particle microfluidic handling, paper-based microfluidic systems still lag behind its more advanced chip-based microfluidic counterpart in terms of analytical sensitivity and multiplexing ability. Long-term stability issues of paper-based assays have also to be addressed.[275]

8. Drug Delivery

Besides oral ingestion, which may not always constitute the best pathway for drug delivery, the delivery of drugs is fundamentally challenging as human physiology has evolved remarkably into an excellent barrier to prevent the entrance of external entities. For example, skin poses a formidable impermeable barrier that protects the body from its microorganism-laden surroundings by restricting the entry of large molecules and hydrophilic compounds. The respiratory system, on the other hand, comprises a highly efficient trap to stop aerosolized material from entering the lungs. Transdermal and pulmonary drug delivery are therefore all but a simple process. With the ability to manipulate fluids and molecules at the micro- and nanometer scales using microfluidic technology, the passage of drugs and therapeutic agents into the human body can be enhanced by various novel means, thus making drug administration more readily assessable, portable, and efficient.

Implantable microfluidics devices and in vivo drug delivery mechanisms can also be useful in cases in which a drug needs to be repeatedly delivered to a patient at frequent intervals. The latter can take the form of biodegradable micro- or nanoparticles, within which drugs or therapeutic molecules are encapsulated, that hydrolyze in vivo therefore releasing its content regularly over time; this is discussed subsequently in Section 8.4. The former consists of implantable microfabricated chambers that release the drug periodically or continuously, and can be refilled if necessary.[276,277] These can be ingested and designed to adhere, for example, to the mucosal membrane or intestinal wall,[278] to allow targeting of the gastrointestinal tissue, injected or inhaled, provided the devices are sufficiently small (typically below 100 μm in dimension). In principle, drug dosing can also be potentially controlled using external stimuli such as ultrasound or microwaves to locally heat and hence activate molecular processes[279] and even DNA hybridization via inductive coupling,[280] although further engineering is required before this can be carried out controllably and practically in vivo. 'Smart' devices which monitor and analyze physiological signals within the body and regulate the drug payload accordingly are already being envisioned and are expected to form the advanced implantable drug delivery systems of the future.

8.1. Transdermal Delivery

Transdermal delivery encompasses methods that enhance penetration of drug molecules through the skin's outer layer, i.e., the stratum corneum, to allow passage into the dermis in which the blood capillaries reside such that they can be absorbed by the blood stream. Transdermal delivery has many advantages over intravenous administration, not least constituting a painless delivery method which eliminates the risk of disease transmission through syringe reuse. Moreover, transdermal delivery constitutes a non-invasive delivery vehicle that permits self-administration.

Since its origins in the form of the transdermal patch, transdermal delivery research has primarily been focused

on utilizing microscale mass transport to increase the permeability of the skin and/or to enhance the driving force for molecular transport through the skin.[281] One approach is to use chemical enhancers to either disrupt the lipid bilayer in the stratum corneum via the introduction of amphiphilic molecules into the structure,[282] or to solubilize and hence increase partitioning of the drug into the skin,[283] although skin irritability can often be an issue. More recent developments have involved the use of peptide enhancers.[284]

Electric and acoustic fields can also be used to disrupt lipid bilayers through electroporation and ultrasonic processes, respectively. An example of the former is a study to demonstrate transdermal peptide delivery,[285] whereas the latter has been adopted to deliver a variety of drugs such as steroidal or nonsteroidal anti-inflammatory agents, hormones, and anti-retrovirals into the skin.[286,287] In both cases, caution has to be employed to avoid pain or damage of the tissue below the stratum corneum. In electroporation, this requires careful placement of the electrodes in a manner such as to confine the electric field in that region whereas in ultrasound, this involves limiting the irradiation levels. Excessive irradiation levels can also result in drug denaturation.

Another approach is to employ iontophoresis, in which an electric field is used to provide a driving force to push charged drug compounds electrophoretically through the skin and to transport uncharged compounds via electro-osmotic action of water, the rate of which can be adjusted through the level of current applied.[288] This is particularly attractive as the delivery can be adjusted as desired, although large currents have been known to cause some discomfort or to result in the denaturation of the drug. In a process known as reverse iontophoresis, molecules can also be extracted transdermally, which was shown to be useful for blood glucose monitoring.[289]

Microfabricated needles represent another vehicle for transdermal delivery. Although minimally invasive in procedure, in contrast to the abovementioned methods, microneedles offer a pain-free alternative to parenteral adminstration such as intravenous and intramuscular injections. By piercing the stratum corneum, skin permeability can be increased. Moreover, the microneedles can be coated or encapsulated with the drug to serve as a carrier for transport during their insertion.[290] A considerable amount of research has been carried out to alleviate concerns of needle breakage either during insertion or due to shear forces exerted by movements along the skin's surface. This has culminated in various designs in the needle configuration and construction materials. These designs are also important in minimizing clogging of the needle.[291] In addition, micropumps, check valves, and flowmeters as well as neural stimulation and recording probes have been incorporated in more complex variations of integrated microneedle delivery devices.[292]

8.2. Pulmonary Delivery

The easy access to the large surface area of the lung, its thin epithelium lining and highly vascularised nature makes it ideal for non-invasive systemic drug delivery. While inhalation therapy or pulmonary drug delivery, which involves the uptake of liquid droplets or solid particles sufficiently small to be suspended in air, constitutes the simplest and most straightforward manner in which therapeutic agents are delivered to the lung, aerosolization is fundamentally difficult because attractive intermolecular forces binding molecules that make up a compound or solution need to be overcome to create the new particle or droplet surfaces. The energy that is required to perform this task is related to the difference in surface energy of the parent liquid or solid compound and the atomized droplets or particles, and is proportional to the increase in surface area associated with the atomization process. Generally, a uniform optimum respirable droplet or particle size between 1 and 5 μm is desired for systemic administration of the drug to the lung, therefore requiring large surface energies and demonstrating the inherent difficulty of the process. This specific size range arises because droplets of these sizes are more likely to navigate the highly bifurcated airways of the respiratory tract in order to be deposited within the 'deep' lung region.[293]

There are a number of conventional methods for aerosol droplet and particle generation. Drug particles are normally produced by large-scale multistep bulk procedures involving batch crystallization, filtration, drying, and milling, and administered using dry powder inhalers (DPIs).[294] Droplet aerosols, on the other hand, can be simultaneously generated and administered using pressurized metered dose inhalers (pMDIs) or nebulizers.[293] Microfluidic technology applied to pulmonary delivery affords a number of improvements over such conventional technology through a number of ways. Miniaturization of the aerosolization process onto a chip-based device, for example, allows simultaneous production and delivery of drug particles, as well as providing a more efficient, automated, precise, and controllable means for monodispersed droplet generation within the optimum respirable size range, to constitute a low-cost portable platform for inhalation therapy. Examples of microfluidic nebulization and particle delivery are discussed below.

Nebulization, which employs an external energy source to overcome the capillary stress and hence destabilize the interface of the parent liquid volume containing the drug solution or suspension, has many advantages over the conventional inhaler technologies that typically dominate the current inhalation therapy market. Because inhalers require breath-actuation that is coordinated with the respiratory uptake, patient training on correct usage technique is necessary. This, however, poses a challenge for inhaler use with infants, the elderly and those that suffer from severe forms of chronic obstructive pulmonary disease, resulting in a high level of misuse. Nevertheless, conventional nebulization technology has not been sufficiently efficient in the past to justify the replacement of pMDIs or DPIs despite their inherent limitations. This was primarily because jet nebulizers often required bulky compressors and were fairly inefficient whereas ultrasonic nebulizers were large and expensive.[293] Novel microfluidic technologies designed to circumvent the drawbacks associated with conventional nebulizers could however bring about a re-emergence of nebulization technology in the inhalation therapy market. Some of these technologies are discussed next.

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8.2.1. Electrospraying

Electrohydrodynamic atomization or electrospraying is a means for generating a monodispersed distribution of aerosol drops in the optimum size range. In particular, DC electrospraying has been demonstrated for the aerosolization of methylparahydroxybenzoate, an antifungal and antibacterial agent used as a preservative in pharmaceutical preparations, and beclomethasone dipropionate, a glucocorticoid steroid used for the treatment of allergies and asthma.[295] The simplicity of the setup, which simply consists of a liquid reservoir feeding into a capillary nozzle connected to a high-voltage source, facilitates miniaturization of the technology into a cheap and portable consumer device that has so far evaded the majority of conventional nebulization technologies. There are, however, some disadvantages with the use of DC electric fields that have prevented widespread adoption in the consumer market.[120] Due to charge accumulation in the conical menisci (more commonly known as Taylor cones), the ejected aerosol droplets possess net charge, which can lead to undesirable surface adsorption effects in the respiratory tract or lung. The incorporation of a charge neutralization mechanism, such as corona discharge, is necessary, but could be prohibitive for miniaturization efforts. The large-kilovolt-order DC voltages also pose significant hazards for use in commercial devices and could also result in the denaturation of drug molecules. The electrical conductivity of the liquid also has to be sufficiently high for DC electrospraying but not excessively high that it results in the onset of spray instabilities;[20] such a narrow window of liquid conductivities places considerable constraints on the number of drug solutions that can be electrosprayed.

High-frequency (>10 kHz) AC fields[121,123] can however be used in place of DC fields to circumvent these limitations. Unlike in DC electrospraying, droplets produced using AC electrosprays do not possess net charge. The electroneutral droplets also minimizes the current through the electrospray, which, together with the short field-reversal periods compared to hydrodynamic shear relaxation times, prevents lysing of drug molecules. The low current also means that the power required for electrospraying is small, thus allowing smaller high-voltage power supplies to be used in the attempt to miniaturize the device. Moreover, high-frequency AC fields are inherently safe.[120]

8.2.2. Surface Acoustic Waves

The large substrate acceleration imposed by a SAW on a liquid drop, on the order of 10 million *g*'s, is a highly effective mechanism for interfacial destabilization leading towards atomization and comprises another method for efficiently generating aerosol droplets.[21,151] Unlike electrospraying, which involves large voltages, and conventional ultrasonic atomization, which necessitates bulky and cumbersome transducers requiring tens of watts of power,[293] the efficient fluid-structural coupling of the SAW permits atomization to be carried out with only around 1 W, which is sufficiently small to be powered by small camera or watch batteries. Hence, the SAW nebulizer can be packaged into a low-cost compact portable format. Using an in vitro lung model prescribed by the British Pharmacopeia, the SAW nebulizer yielded 70–80% lung dose efficiencies for the pulmonary delivery of the short-acting β_2-agonist salbutamol, which is a significant improvement when compared with the 20–30% efficiencies typically obtained using currently available off-the-shelf inhalers.[296] The high frequency of the SAW and the low power required also suppresses cavitational damage and minimizes shear damage of the drug molecules, both of which are serious concerns associated with the use of conventional ultrasonic transducers.[119,122]

SAW atomization has also been shown to be an effective tool for nanoparticle generation.[297] Due to the rapid generation of a large number of nanoparticles directly from the interfacial destabilization and atomization process, simultaneous particle generation and pulmonary delivery using the same microdevice can be carried out. With disposable fluid loading cartridges, particles can therefore be generated at the point of delivery, thus eliminating stability issues associated with long-term nanoparticle storage. Protein particles between 50 and 100 nm generated in this manner have been shown,[298] both as a one-step method for nanoparticle synthesis and a device for direct pulmonary administration, which is an attractive route for protein and peptide delivery given the low proteolytic activity in the lung compared to other delivery pathways.

8.3. Gene and Vaccine Delivery

The emergence of large-scale genetic sequencing and analysis has heralded a new advent in genetically engineered therapeutics. Gene therapy can take two primary forms, either delivering the gene to germ cells (reproductive cells such as eggs and sperm) for early stage embryonic genomic modification and hence transmission to subsequent generations as a way to correct genetic defects (germline gene therapy), or, transferring nucleic acid material to the somatic cells and hence the tissues (lung, heart, kidney, etc.) of a patient without involving hereditary transmission (somatic gene therapy) in order to increase the expression of genes already present for the production of specific proteins. Although ethically controversial, gene therapy has the potential to treat genetic disorders and could revolutionize the development of vaccines. Microfluidics is already playing a role in gene therapy, for example, as a platform for controlled vector preparation and analysis of recombinant viruses using the various on-chip PCR amplification, enzymatic digestion, and separation tools outlined in Sections 2 and 3.[299]

Here, we categorize gene delivery into two broad classes, namely, the direct insertion (transformation, or transfection in the case of mammalian cells) of genetic material into cells, and, the bulk delivery of genetic material via various administration routes (parenteral, including subcutaneous, intravenous, and intramuscular; oral; pulmonary; nasal; ocular; and transdermal). In both forms of delivery, genes are delivered to target cells through either nonviral (naked plasmid DNA (pDNA), short-interfering RNA (siRNA), or synthetic, e.g., cationic lipids and molecular conjugates) or viral (retroviruses, adenoviruses, adeno-associated viruses, lentiviruses,

herpes simplex virus, etc.) vectors, the latter providing better transfection efficiency but which could limit insertion-lengths. Moreover, even the safest viral vectors such as the adeno-associated viruses, are immunogenic, and have been known to result in patient deaths.

8.3.1. Direct Transfection

Localized gene delivery through direct insertion involves the temporal creation of pore openings in the cell membrane to facilitate the uptake of the linked target and vector into the cell. Several methods for direct transfection exist, some of which such as electroporation and sonoporation have been discussed in the context of cell lysis in Section 4.2.2, though the former, which only requires the use of patternable electrodes and low voltages, is more amenable for integration into a microfluidic device.[300,301] In electroporation, the transfection efficiency depends on pulse duration and intensity, as well as cell size, and DNA concentration and conformation. Optical transfection, on the other hand, employs nanosecond or femtosecond pulsed lasers, or continuous wave lasers. Short-pulsed optical fields generate free-electron plasma at the cell surface, which causes the opening of the pore whereas the longer nanosecond pulses and, more so for the continuous wave lasers, involve transient thermoelastic pore cavity formation. While it has been claimed that optical transfection is compatible with microfluidic sandwich coverslip formats, the necessity for large lasers places complete on-chip integration out of reach at least in the near future.[302]

Magnetic fields, in contrast, can also be employed for transfection with high efficiency, in which they are used to drive the sedimentation of polyethylenimine-coated superparamagnetic iron oxide gene vectors known as magnetofectins on the cell surface to enhance their endocytic (cellular ingestion of genetic material through membrane folding) uptake,[303] although it is yet to be shown whether this can be carried out on a microfluidic chip. On the other hand, heat shock processes, in which the cell is subject to a sudden change in temperature, usually around 42 °C, can also induce transfection and has been integrated into a chip format.[304,305]

Perhaps the most straightforward way to incorporate the gene transfection process into a microfluidic platform without electrodes, heaters, or ancillary equipment such as magnets or lasers, however, is to employ chemical transfection agents, for example, cationic liposomal and polyamine based reagents.[306] This has also been integrated into a high-throughput live cell microarray chip for the screening of potential small-molecule drug targets, whose binding indicates the overexpression of a specific receptor as an alternative to protein microarrays (Section 3.1), which require the purification of a large number of proteins. In this particular case though, 'reverse transfection' was carried out in which the order of DNA and cellular addition was reversed.[307] To localize the transfection in the absence of physical confinement, the pDNA were immobilized in the gel where it can only interact with neighboring cells. A similar reagent-based transfection microarray device, but for transfecting human mesenchymal stem cells, has also been developed.[308] Whichever the case, the choice between the transfection methods listed above nevertheless depends on the genetic material to be transfected and the type of cell it is transfected into.

8.3.2. Bulk Gene Delivery

Microfluidic systems can be exploited as a delivery platform for gene therapy, in particular the administration of nucleic-acid-based vaccines, which employ the host cell's transcriptional and translational capability to produce the desired protein. Unlike conventional vaccines that employ whole pathogens or recombinant proteins, DNA or RNA vaccines do not contain a viral coating and hence do not invoke antibody reactions that suppress vaccine efficacy. Moreover, in addition to being safe and easy to produce, these vaccines present the opportunity for combining the genetic information of various antigen epitopes and cytokines.[309] Until now, injection has been the most widely adopted administration vehicle for nucleic acid vaccines. Nevertheless, the promise of pain-free, non-invasive and efficacious targeted delivery through the mucosal surface of the respiratory system, which is a less harsh environment than the gastrointestinal tract and which represents the first line of defense against pathogens entering via the inhalation route, using microfluidic technology renders pulmonary delivery a potentially attractive immunization pathway to induce systemic immunity. In addition, there has been significant interest in targeted gene delivery to the lungs using aerosols, primarily for the treatment of pulmonary diseases such as cystic fibrosis, pulmonary alveolitis, and lung cancer.

While inhalers are perhaps the most logical starting platform for aerosol gene delivery, microfluidic nebulizers offer the benefits of miniaturization, improved delivery efficiency and reduction in waste discussed previously in Section 8.2. Moreover, the potential to precisely alter the droplet size and flow rate offers the ability to optimize the system for varying patient physiological profiles or need, the latter by targeting delivery at specific regions of interest. For example, larger aerosols above 5 μm tend to deposit on the upper respiratory airways or oropharyngeal regions and can be used to treat upper respiratory tract infections, whereas smaller particles below 3 μm deposit in the distal and terminal airways and can be used to vaccinate against lower respiratory tract infections.

Before any technology can be approved for use, however, the integrity of the DNA has to be demonstrated. The large shear stresses generated by conventional mechanical and ultrasonic nebulizers which leads to possible fragmentation of the supercoiled structure in the case of pDNA-based approaches and large losses in transfection efficiency[310] can be prevented with the use of high-frequency systems such as the AC electrospray[120,121,123] and SAW atomizer.[151,296] In fact, promising results have been demonstrated though imaging of post-SAW-atomized pDNA as well as in in vitro transfection studies analyzing MSP4/5 (mouse malaria merozoite surface protein) gene expression of the recovered pDNA in African green monkey kidney (COS-7) cell lines compared with the corresponding unatomized pDNA.[311] pDNA stability during nebulization can be additionally enhanced, for example, by complexation with cationic polymers such as

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dendrimers, chitosan or polyethyleneimine (PEI). The latter, in particular, has been shown to increase lung reporter gene expression by 15-fold in in vivo mouse and sheep lungs without generating inflammatory responses.[312]

Given that skin consists of immunologically rich tissue, transdermal delivery (Section 8.1) offers an attractive delivery route for topical immunization, which can be as or even more effective than parenteral routes as long as the antigen and adjuvant can be transported to the dermis. For example, microneedles can be used to pierce sufficiently large holes through the stratum corneum to allow pDNA to pass through. This has been demonstrated in mice for immunization against the hepatitis B surface antigen.[313] Another place where microfluidics can play an important role in gene delivery is through the patterning of DNA complexes on cell adhesive substrates as a way to provide spatial control for directed cellular migration and differentiation. In this work, the DNA complexes, immobilized using the same plasma treatment employed for the deposition of protein patterns in PDMS microchannels, are shown to result in gene expression of the neurotrophic nerve growth factor that is capable of regulating neurite growth over 100 μm length scales.[314]

8.4. Drug Encapsulation and Targeted Particle Delivery

The encapsulation of DNA, peptides, proteins and other therapeutic molecules (e.g., growth factors, hormones, antibodies, antigens, etc.) within biodegradable polymeric excipients is vital for the controlled and targeted ophthalmic, oral, intravenous, or implanted delivery of vaccines and drugs for manifold purposes. Firstly, the encapsulation shell provides a shield that isolates these substances from hostile environments, thus preventing their susceptibility to decomposition, enzymatic degradation, aggregation, and denaturation, thereby prolonging their half-lives in vivo as well as their shelf life. Moreover, hydrophobic compounds can be encapsulated within amphiphilic capsules to allow injection into the blood stream. Secondly, the molecular diffusion through the shell, which itself biodegrades, can be controlled, therefore preventing initial bursts of dosage and thus providing a means for the slow controlled release of the drug over time. For patients who depend on daily medication, this could prevent sudden initial bursts of dose during administration, which could be dangerous with medication such as insulin. Finally, the specificity of the drug can be engineered to avoid uptake by the liver, spleen, or other parts of the reticularendothelial system and to locally target the diseased lesion or tumor by judicious choice of the polymer with different characteristic surface properties or through modification of its surface functionality. This last purpose also demonstrates the exciting possibility to design specific drug delivery systems for individuals, which could represent the future generation of drugs.[120]

While there are various conventional routes to achieve encapsulation such as polymer solvent extraction from a double emulsion through evaporation, phase separation (coacervation) or spray drying, these bulk laboratory-scale multistep methods are cumbersome, slow, and do not permit simultaneous in situ encapsulation and delivery. Microfluidic encapsulation strategies provide a remedy to these limitations in a quick one-step straightforward manner that requires little sample handling and manual laboratory intervention. Using either DC and AC electrospraying or SAW atomization of a biodegradable polymeric excipient dissolved in a suitable solvent, in which the compound to be encapsulated is dispersed, the evaporation of the solvent in-flight leaves behind a micron dimension solidified spherical polymer shell within which the encapsulant is trapped.[120,315,316]

An alternative method for encapsulation using DC electrospraying has also been demonstrated although this setup required the use of two co-axial needles. The inner needle is used to electrospray the aqueous liquid in which the encapsulant is dispersed, and is surrounded by an annular sheath of organic liquid solvent and photopolymer confined within the outer needle. To solidify the photopolymer, UV light is passed through the electrospray droplets.[317] In addition to the necessity of a photopolymer, another disadvantage of this setup is the requirement of the encapsulant to be dispersed or dissolved in the aqueous phase, which precludes a large number of organic soluble drug compounds.[120] Moreover, it not clear whether the molecular structure of the encapsulated drug is preserved under the large currents and high applied voltages required with DC electrospraying. Little shear degradation or lysis of the encapsulant is however encountered with AC electrospraying or SAW atomization due to the high frequency of the electric and acoustic fields beyond that of the molecular shear relaxation frequency.[119–122] Besides, the fabrication of the co-axial structure requires precise machining and assembly, which limits mass production and widespread application of the technology.

Encapsulation can also be carried out on-chip in a microdevice using a droplet microfluidic platform.[170,171] The same technique used to encapsulate individual cells within a train of aqueous droplets surrounded by an immiscible oil medium inside a microchannel for single-cell analysis[236] (Section 4.3.2) can be employed to capture cells, proteins and other therapeutic molecules, for example, within lipid vesicles.[318] Double (e.g., water-in-oil-in-water or oil-in-water-in-oil) emulsions, which can be used for sustained or prolonged delivery of the drug payload, can also be generated in a similar manner using multiple T-junctions to sequentially inject one immiscible phase into another (**Figure 19**).[319]

Micro- or nanometer dimension polymer multilayer capsules can also be synthesized to achieve further control of the drug release. By carefully selecting different polymers comprising each layer according to their binding characteristics with the drug (e.g., hydrogen bonding, hydrophobic interaction) and their degradability in solution, the desired release profile, which could even involve transient variation, can be achieved. Further control can be obtained by tuning the thicknesses of each layer, the number of layers and the mass ratio between the polymers comprising each layer. Traditionally, encapsulation within successive oppositely charged polyelectrolyte multilayers has been carried out using layer-by-layer assembly,[320] which involves the alternate and consecutive deposition of complementary and interacting polymers onto colloidal templates, after which the template itself is sacrificially removed. The choice of pH and salt concentration used

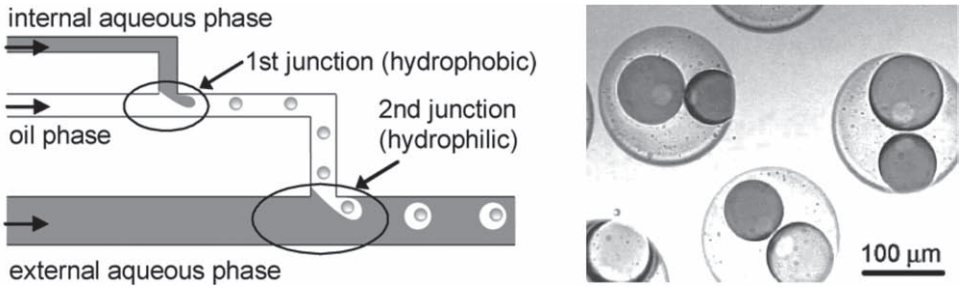

Figure 19. The left image shows a schematic illustrating the method in which double (water-in-oil-in-water) emulsions can be produced in a droplet microfluidic system. The right image shows the encapsulation of two aqueous droplets in a parent oil droplet. Reprinted with permission.[319] Copyright 2004 American Chemical Society.

during the assembly also facilitates further control over the drug release. More recently, a fast and simple alternative for synthesizing and encapsulating drugs within polymeric multilayer capsules has been shown by successively atomizing one polymer solution into another using SAWs.[321]

9. Biomaterials Synthesis and Tissue Engineering

Tissue and organ transplantation is now an accepted and widely used therapy for the treatment of damaged or defective tissues and organs. Nevertheless, transplant surgery is not only extremely costly but can involve high risks due to possible complications. Long wait times are also common due to the perennial shortage of suitable donor tissues/organs. Tissue engineering, in which a patient's own cells can be grown within biodegradable and biocompatible 3D scaffold matrices and subsequently implanted in vivo to synthesize replacement tissues or organs, is a promising alternative which could potentially alleviate inadequate donor tissue or organ supply. There are three basic steps for the engineering of tissues, first involving the expansion of cells from a small biopsy, then culturing the cells in vitro within temporary 3D scaffolds to form the new extracellular matrix, and finally implanting the cell and scaffold composite in vivo to repair the defective tissues or organs. Tissue engineering constructs can deliver a patient's own cells, thus negating the need for allograft transplantation and the immunosuppressant regimen to sustain allograft tissue, and alleviating the problems associated with organ or tissue donation shortage or the serious immunological problems commonly observed in many transplants. Moreover, implanted scaffolds will degrade within the body, which eliminates the need for subsequent surgery to remove the implants.

9.1. Bioreactors and Microarrays

Microfluidics can play several roles in enabling tissue or orthopedic engineering, or in aiding the process to be more efficient in practice, particularly by providing the enabling strategies that facilitate the assembly of cells to synthesize primitive tissue structures as well as the tools by which these structures can be remodeled, for example, by spatiotemporally manipulating the local cellular microenvironment to encourage tissue morphogenesis and differentiation.[322] Compared to conventional 2D monolayer cell culture, 3D tissue engineering constructs impose more stringent requirements on the exchange of metabolic products through the continuous supply of oxygen and nutrients, and the removal of waste products. The capability to fabricate microfluidic perfusion networks as well as to amalgamate microvalves, microactuators, and micromixers therefore allows such mass transport limitations associated with conventional bioreactor technology to be circumvented. This is augmented by the ability to automate and integrate microfluidic controls and sensors for the spatiotemporal regulation of the cellular biochemical and mechanical microenvironment (see Section 4.1). Long-term cell culture can also be conducted since cellular densities are significantly higher than that obtained with conventional static culture.

Microfluidics, in addition, provides a means for interconnectivity between multiple compartments in microscale bioreactors for the co-culture of several cell lines that share the same environment such that cell physiology and metabolic pathways can be verified more accurately and efficiently.[323] In fact, current microfluidic bioreactor technology with interconnected compartments proportional to physiological architectures has now advanced to a point where there has been some success in mimicking the response of multiple animal and human organs, such as the lung, liver, and fat tissue, to chemical toxicity. This is particularly useful as in vitro models for pharmaceutical trials[324]—for example, a microfluidic hepatocyte culture system was developed to mimic the human liver, from which hepatic clearance data can be obtained.[325]

Microfluidic bioreactors in cell microarray format also play an important role in tissue engineering, for example, to facilitate clonal analysis by localizing and tracking individual stem cells to determine their fate during culture.[326] Such tools are also extremely useful for enabling high-throughput screening libraries, which, in the context of tissue engineering, can be used to identify targets to induce cardiomyogenesis and osteogenesis.[327] In addition, we have discussed in Section 4.1 the role of topological cellular patterning and other extracellular cues on controlling the fate of stem cells, in controlling apoptosis and proliferation and in directing differentiation pathways, and how these can be regulated using cell microarrays.

Cell patterning and assembly using microcontact printing and templating can also be used to reproducibly synthesize

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artificial microtissue structures without requiring scaffolds. Such scaffold-free synthesis is attractive not only because it is simple and straightforward but also since natural assembly forces do not interfere with cell regulation, thus resulting in tissue growth that is not hindered by limitations in oxygen and nutrient supply.[328] In addition, scaffold biodegradation can also lead to inflammatory reactions.[329] Microtissue structures, although lacking in sufficient complexity to provide a route towards engineering larger artificial organs, may still be useful, for example, in repairing damaged scar tissue due to injury or neurodegenerative/cardiovascular disorders where self-repair is no longer possible. The key to the synthesis lies in the assembly of cells in such a manner as to naturally induce the monodispersed cells to reaggregate in a way that mimics in vivo tissue growth. While gravity-driven reaggregation using gyratory shakers, spinning flasks, and pendant drops can be employed,[328] micropatterning and template-based assembly methods provide a controllable and reliable way to reproducibly synthesize microtissue and can incorporate measures for organizing multiple cell types or altering cell differentiation.[327] Alternatively, we envisage microfluidic technology to be an extremely powerful tool to layer sheets of cells onto support membranes to reconstruct tissue structure. This has been demonstrated using cardiomyocytes, which when confluently cultured onto sheets, connect via gap junctions to mimic the simultaneous beating of native heart tissue.[329] Sheets of human vascular smooth muscle cells rolled around a tubular support into a cylinder have also been engineered into blood vessels.[330]

9.2. Scaffold/Fiber Synthesis and Cell Seeding

Microfluidics can also be employed to synthesize fibrous biomaterials and scaffolds and to print or seed cells onto or into these scaffolds to achieve the desired spatial distribution or to provide the appropriate external stimuli for cell differentiation within the scaffold. Further, with the ability for tuning the scaffold properties during microfluidic synthesis, it is possible to exert additional control over cell growth—for instance, it has been shown that a 3D hydrogel scaffold can stimulate the neurogenesis of human mesenchymal stem cells in the absence of chemical stimuli simply through the substrate stiffness.[331]

In addition, similar microfluidic strategies to that discussed in Section 8.4 for the encapsulation of therapeutic agents within biodegradable polymer matrices for drug delivery can be utilized to embed growth factors for controlled delivery to specific tissue sites to repair damaged cells or to stimulate cell migration and proliferation. Controlled delivery can also be achieved using the microneedle technology described in Section 8.1. Similar microcapillary array tips have been demonstrated for local delivery, for example, of gene-containing pDNA into cells (see also the discussion on gene delivery in Section 8.3). Alternatively, multichannel silicon probes have been employed to target localized regions of brain tissue with stimulants to modulate neuronal activity while simultaneously measuring electrical signals given off by the neurons.[332] Such local delivery can also produce concentration gradients of growth factors within the tissue matrix, which have been observed to have an effect on embryonic tissue development. Microfluidics can also be exploited to provide temporal concentration gradient control as well as spatial patterning by allowing adjustments in the rate at which the drug is delivered.[333]

The synthesis of scaffold constructs and fibrous biomaterials has emerged as an important technology given its relevance to tissue and orthopedic engineering, wound healing therapy, and vascular grafting. These biomaterials function as a 'skin' for implanted nonbiocompatible devices made of materials that would otherwise trigger an undesirable inflammatory response. There have also been collective efforts to develop highly porous bioscaffolds that progressively imitate the structure and function of the in vivo ECM of tissues or organs, thus providing mechanical support to the cells and maintaining the organizational definition of the tissue space while preserving its biocompatibility and reabsorbability.

9.2.1. Direct-Write Methods

A number of microfluidic synthesis methods are available as an alternative to colloidal templating or solid free-form fabrication (rapid prototyping) approaches such as polymer replica molding, micromachining, photolithographic patterning and writing, 3D printing, or stereolithography. The advantage, however, offered by 3D printing, and, in particular, microfluidic direct-write assembly, for example, lies in the ability to control the spatial patterning of a variety of materials with arbitrary shapes over very small micron length scales in a simple manner. In direct-write techniques, the pressure-assisted deposition of polyelectrolyte inks, polymer solutions, or hydrogels onto a moving x–y stage can be achieved by mounting the syringe to a z-axis drive and printing 2D layers in stacks to form a 3D vascular scaffold network. The ink network can then be injected with an epoxy resin and cured, following which the ink is extracted. Alternatively, the polymer or hydrogel can be solidified via photopolymerization.[334] Internalized structures with arbitrary connectivity within the 3D scaffold network can also be patterned by injecting the scaffold with a photocurable polymer, subsequent mask alignment followed by UV exposure.[335]

In place of pressure-assisted syringes, electric fields can also be employed. Electrospinning is essentially the process of electrospraying higher concentrations of polymeric or protein solutions or even sol–gels such that fibers ranging from several microns down to 100 nm are extruded either from the tip of the DC Taylor cone or generated due to extensional stresses from the meniscus under an oscillating AC field.[20,120,336] As in direct-write methods, the electrospinning nozzle can be attached to robotically controlled stages to achieve 3D scaffold architectures with topologies such as nanoporous surfaces, crystalline geometries, core–shell configurations, beads, hybrid natural and synthetic blends, or multistranded threads that have high surface-area-to-mass ratios to facilitate cell attachment, support cell growth, or regulate cell differentiation.[336–339] In addition, therapeutic agents can also be encapsulated within the fibers for controlled release and gene delivery[340] (see also Sections 8.3 and 8.4), although

biomolecular or cellular lysis due to the large DC electric fields remains a concern. Another limitation of electrospinning is the requirement for organic solvents within which the polymeric excipient is dissolved.[341] Little attention has also been paid to simultaneous cell seeding within synthesized fibers despite the preference of in situ cell seeding during scaffold synthesis over subsequent cell seeding into preformed scaffolds due to the difficulty of achieving homogeneous loading in the latter (discussed below). Nevertheless, we anticipate new technologies for direct-write scaffold synthesis and encapsulation as well as cell seeding and handling to emerge from the recently discovered SAW jetting phenomenon,[139] which, unlike its piezoelectric ink jet predecessors, does not suffer from limitations due to nozzle clogging, large droplet and fiber diameters, large and bulky equipment, and the tendency for shear- or cavitation-induced biomolecular degradation.

9.2.2. On-Chip Microfluidic Systems

Direct-write processes nevertheless involve complex and expensive robotic control and often laborious pixel-by-pixel writing. On the other hand, however, it is possible to pattern scaffolds layer by layer using microfluidic channels within which the polymer matrix and cells are sequentially flowed through and deposited to create a 3D structure that permits spatial regulation of the cellular microenvironment.[342] The fabrication of solid foam gel scaffolds as a means to culture chondrocytes was also demonstrated using a microfluidic device that facilitated the axisymmetric concentric flow of nitrogen gas within an aqueous alginate solution and surfactant.[343] Here, the controlled generation of bubble trains within the alginate in a similar fashion to a droplet microfluidic platform[170,171] is exploited to produce a regular and monodispersed array of bubbles in the alginate, which is then gelated to produce a honeycombed solid foam scaffold structure. A number of other photopolymerizable hydrogels have also been used in conjunction with microfluidic systems since the technology offers the ability for spatial regulation by varying the cross-linking density or by inserting soluble signaling factors in a manner to form concentration gradients across the hydrogel such that cell migration, adhesion and differentiation as well as axon extension and angiogenesis can be controlled.[327,344–346] In addition, cells can also be embedded into hydrogel microchannel structures by adding cells during molding.[347]

9.2.3. Incorporating Vasculature in Scaffolds

Without sufficient vascularization into the scaffolds provided through a capillary network, tissues are unable to grow beyond a certain thickness due to the mass transfer limitation of oxygen and nutrients into the scaffold. Consequently, the engineering of large and complex functional organs for clinical use remains out of reach. Given the micron-dimension of the vascular capillary network, however, microfluidic technologies are appropriately positioned to address this limitation. Initial attempts in building a planar vascular network into a scaffold structure utilized soft lithography to mold a PDMS layer containing a bifurcated microchannel network onto a silicon wafer.[348] An important criterion in the selection of the design is to ensure flow uniformity while minimizing hydrodynamic resistance. In addition, the magnitude of the shear stress imposed in the microchannel vascular network has been observed to play an important role in the endothelialization process and hence in angiogenesis.[349]

Another important factor is the biodegradability of the material—with this in mind, artificial microvascular networks can also be fabricated from biodegradable polymers, which can also be bonded and stacked to form a multilayer 3D monolithic network.[350] In addition, the use of direct-write assembly to synthesize a 3D microvascular network has been discussed in Section 9.2.1.[335] Synthetic polymers, while biodegradable, however, can often produce cytotoxic by-products and invoke undesirable inflammatory responses during implantation. As discussed previously, hydrogels can also be fabricated into a 3D channel network which allows efficient mass transfer in and out of the scaffold.[346]

9.2.4. Cell Seeding Within Preformed Scaffolds

Microfluidics also offers the opportunity to enhance cell seeding within scaffolds that are prefabricated using other methods. Although conceptually simple, the process is nontrivial as any application of external driving forces could potentially result in the denaturing of the cells. In addition, efficient seeding involves achieving a homogeneous cell distribution, which can be difficult given the complex microarchitecture of the scaffold interior and the large capillary stresses associated with the flow through the micron pore dimensions. Static seeding, which relies on gravity perfusion to draw the cell suspension through the scaffold pores is not only slow but also woefully inadequate, with only superficial penetration being achieved; consequently, cells only proliferate around the periphery of the scaffold. Indeed, studies have reported that new bone tissue is easy to form at the surface of scaffolds but difficult to regenerate at the center of porous scaffolds because more cells collect on the surface of the scaffold than in the inner part of the structure, even with the use of perfusion bioreactors. Moreover, cells seeded close to the periphery are also prone to be lost upon hydration of the material.

One possibility is to drive the flow of a cell suspension through the annulus of a tubular scaffold.[351] While this significantly reduced the seeding times from typically hours and days to several minutes and resulted in greater distribution uniformity, the use of vacuum is not only expensive but also prohibitive for further miniaturization. Another possibility is to employ SAW microfluidics to drive a droplet comprising the cell suspension into the scaffold.[352,353] This is not only extremely fast, with seeding completed in under 10 s, but also results in a significantly greater and more uniform penetration than other conventional seeding methods; moreover, the entire process can be carried out in a microfluidic chip without requiring large ancillary equipment.

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10. Summary and Outlook

A recurring theme that has become evident throughout this entire review is device integration for true on-chip functionality without requiring large and costly external ancillary equipment such as power supplies, capillary pumps, lasers, and mass spectrometers. Before microfluidic devices can be practically used and more widely adopted in a variety of laboratory, commercial, military, or philanthropic settings, there is a need for full-scale incorporation and automation of complete microfluidic components that carry out a process from start, for example, injection and preparation of the sample, to finish where the desired end product or analytical result is obtained; all of this at costs acceptable to manufacturers and consumers. This will require a genuine collaborative and interdisciplinary effort, spanning from our own efforts in fundamental fluids physics, which underpin efforts to carry out all fluid propulsion and particle manipulation operations (including the power supply) within the microdevice itself right through to endeavors to miniaturize spectroscopic operations for biomolecular detection and characterization.

A more immediate and achievable near-term goal is perhaps the opportunity afforded by seamlessly interfacing microfluidic devices with conventional macroscale laboratory processes through modular 'plug and play' platforms—the Agilent Nano LC/MS which integrates a HPLC chip with mass spectrometry through an ESI-MS interface, is one prominent example. On-chip optical waveguides, which exploit the laminar flow of a fluid that forms the optical core between two cladding fluids in a microchannel, for example, also constitute a promising interim arrangement for optical interfacing; in these waveguides, the properties of the laser can be selectively tuned simply by adjusting the refractive indices of the core and cladding fluids.[354] Further work, however, is necessary to design suitable interfaces for fluid transfer into, within, and out of the microfluidic chip without losses as current techniques, which, for example, include the use of complicated valves and pumps to fabricate large arrays of microchannels that can be individually addressed, may not be entirely satisfactory especially for portable and field-use systems.

Finally, there is the issue of 'user opinion.' One of the reasons for the slow uptake of microfluidics from a broader commercial perspective is a poor understanding of the advantages afforded by microfluidics beyond workers in the field. This has vastly improved from the time microfluidics originated, but there is still a long way to go before such benefits are appreciated by the business manager, who makes the financial decisions on whether or not to invest in the research and development along the commercial pathway towards production, to the consuming public, who place demands on the need for the product itself. There is therefore a need to engage the public, and the commercial interests surrounding it, to enable them to see the benefits of the microfluidic paradigm of doing things in a new and different way, as well as to aid them in overcoming their difficulties in translating their use of current large-scale laboratory equipment which they have invested in for daily routine tasks to new microfluidic systems. Although this seems trivial enough, consider the amount of inertia that was faced (and that is still encountered!) even within the scientific community in trying to convince one's own colleagues outside the microfluidic field to adopt microscale systems in their work.

The outlook, however, is promising, and we envisage all kinds of microfluidic devices, from implantable systems to monitor a range of dynamic physiological chemical and biological processes in vivo and swimming microbots that target the delivery of drugs to a specific site to the synthesis of fully functional artificial organs and personalized diagnostics based on genetic analysis. However and whenever we get there aside, the journey towards this lofty goal will nevertheless be an extremely exciting and challenging one, promising to revolutionize various aspects of science and engineering along the way.

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