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The Analytical Approach to Polydimethylsiloxane Microfluidic Technology and Its Biological Applications

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This review article discusses PDMS (polydimethylsiloxane) microfluidic devices and their biological applications. First, the already developed devices are classified from the viewpoints of underlying technology within a common logical framework comprising single-layer, multilayer, and integrated devices, as well as surface chemistry modifications of PDMS. Combinatorial techniques are applied to re-derive existing devices within this framework. Next, the relevant scales of both microfluidics and biology are compared, obtaining the promise and limitations of PDMS microfluidics. Finally, the body of work is reclassified in terms of addressed biological applications and compared to the standard methods in cellular and molecular biology, to offer insights for future devices and applications.

Keywords: Microfluidic Technology, Polydimethylsiloxane, Biological Applications.

CONTENTS

1. INTRODUCTION

In less than a decade of existence, PDMS (polydimethylsiloxane) microfluidics developed by Whitesides and colleagues at Harvard has negotiated the long distance from the plain channel[1] (Fig. 1) to a plethora of specialized components organized by the thousands in large-scale-integration devices[2] (Fig. 2) developed by Quake and colleagues at Caltech, thereby fulfilling Richard Feynman's dreams of infinitesimal machines[3,4] at least at the micro scale. The now mature technology has already been successfully used in a number of important applications, e.g., protein crystallization,[5] DNA sequencing,[6] nanoliter PCR,[7,8] cell sorting and cytometry,[9,10] nucleic acids extraction and purification,[11] immunoassays,[12–18] and cell studies[19–23] (Fig. 3).

In the present review, we first examine the field from the viewpoint of underlying technology. We apply inductive and deductive methods to retrace the morphological and functional developments of microfluidics within a new combinatorial framework. Then, we reexamine the field from the viewpoint of applications and classify the already accomplished work in comparison to the current macro methods of the biological sciences, thereby deriving ideas for applications to be pursued henceforth.

2. TECHNOLOGY

PDMS microfluidics appeared almost by accident when it was realized that PDMS replication stamps produced by soft lithography methods[24,25] could in their own right become functional devices when coupled to a flat substrate and used to contain fluids.[1] Thus the stamp grooves became microchannels and the stamp ridges became separators and couplers to the substrate. These simplest devices already offered the advantage of volume miniaturization and carried the promise for more complex functionality by further engineering of the channel geometries and device architecture.

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2.1. Single-Layer Devices

2.1.1. Patterning

Due to its soft-lithography construction, a microfluidic channel is a planar object. Then an obvious development would be to have several channels running in the same plane, in parallel, crossing, converging, diverging, connecting, and separating, like the streets of a city. If the same reagent flows through all channels, nothing particularly interesting happens in the channel itself because the conditions are the same everywhere. To increase complexity, and therefore increase functionality, we need to add

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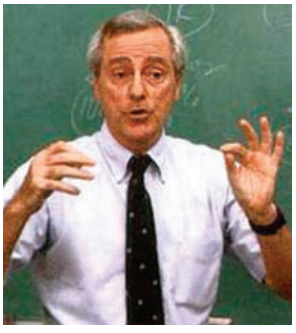

W. French Anderson received an A. B. (magna cum laude) in Biochemical Sciences from Harvard College in 1958, an M.A. (honors) in Natural Sciences from Trinity College, Cambridge University (England) in 1960, and an M.D. (magna cum laude) from Harvard Medical School in 1963. He is the Director of the Gene Therapy Laboratories at the University of Southern California Keck School of Medicine, where he also serves as Professor of Biochemistry and Pediatrics. Before joining the USC faculty in 1992, he was Chief of the Molecular Hematology Branch at the National Heart, Lung and Blood Institute at the National Institutes of Health where he worked as a gene therapy researcher for 27 years. Dr. Anderson headed the team that carried out the first approved human gene therapy clinical protocol in 1990, is recognized as an ongoing innovator in the research area of human gene transfer, and is also known as a leading ethicist in the field of human genetic engineering. He has been called the "Father of Gene Therapy." The focus of Dr. Anderson's present gene therapy research is the development of virus-based vectors to be injected directly into patients for gene transfer; safer, more efficacious vectors for gene transfer into hematopoietic stem cells; and in-utero gene therapy to treat genetic diseases that produce irreversible damage before birth. Finally, Dr. Anderson is also a Visiting Associate in Applied Physics at the California Institute of Technology and is exploring the use of nanotechnology, particularly PDMS microfluidics, for genomic applications and medical diagnostics.

Axel Scherer is the Bernard A. Neches professor of electrical engineering, applied physics and physics at the California Institute of Technology. He received his Ph.D. from the New Mexico Institute of Mining and Technology in 1985 and subsequently worked at Bellcore on the microfabrication of optoelectronic devices. In 1993, he joined the California Institute of Technology and formed a nanofabrication research effort centered on optical, magnetic, electronic, and microfluidic devices. Professor Scherer pioneered the development of vertical cavity lasers and their first large addressable arrays, developed electromagnetic design tools for the definition of high-Q optical microcavities, pioneered silicon opto-electronic devices in the silicon-on-insulator materials system, and miniaturized spectrometers and integrated fluidics into optical devices. Fundamentally new structures, such as the photonic bandgap nanocavities created by three-dimensional microfabrication in Professor Scherer's laboratory have led to the first demonstration of strong coupling between single quantum dots and optical microcavities. This work has resulted in some of the world's smallest lasers, modulators, and waveguides, as well as very-high-efficiency solid-state light emitters based on metal surface plasmons. In his research group, design and fabrication techniques are presently applied towards the miniaturization of integrated microfluidic valves and pumps with optical micro- and nano-devices for nanophotonics and nano-biotechnology.

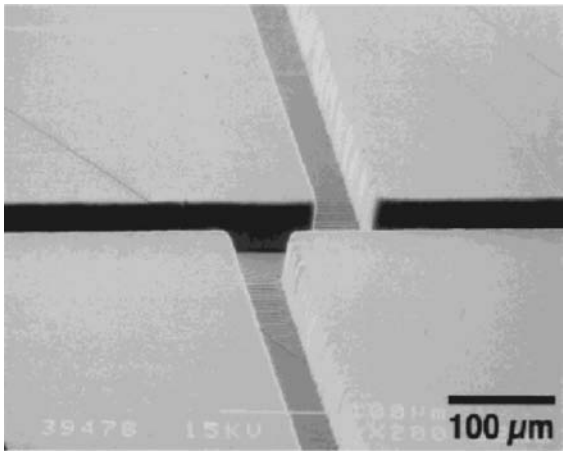

Fig. 1. *The first PDMS microfluidic channels.* SEM images of channels fabricated in PDMS by molding the polymer against a positive relief of photoresist. Reprinted with permission from [1], D. C. Duffy et al., *Anal. Chem.* 70, 4974 (**1998**). © 1998, American Chemical Society.

orthogonal features and thus expand the spanned volume in phase space.

One such orthogonal feature is to allow for surface treatments. For example, one of the surfaces of the channel (e.g., the substrate surface) can be chosen or modified to possess a chemical reactivity that the other surfaces do not. If that reactivity allows the former to bind objects of interest (e.g., chemical species) carried by the fluid in the microfluidic channels, then a parallel network of identical channels delivering identical fluid to different locations on the substrate would deposit the objects on the functionalized surface. Peeling off the chip would leave the objects intact on the substrate in the same geometric formation as the architecture of the channels, thereby producing microfluidic *patterning* of the objects of interest by a 2D network of channels. This capability immediately enables chemical and cell patterning applications.

However, if only one chemical species or type of cell is present in the device, the best we can hope for is multiple copies of the same experiment. It is clear then that

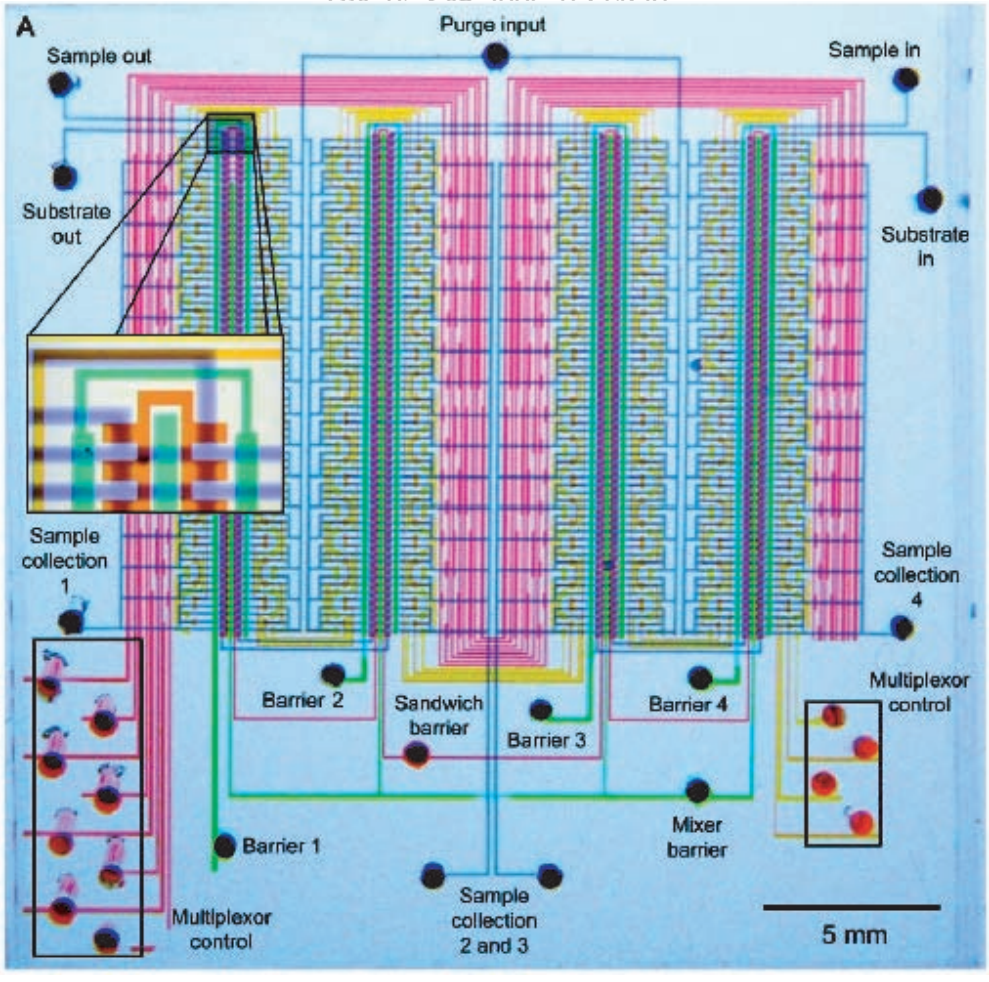

Fig. 2. *Microfluidic large scale integration.* This photo shows an example of large scale integration, containing 2056 microvalves as well as 256 microchambers that can be independently compartmentalized, mixed pair-wise, and selectively purged. Reprinted with permission from [2], T. Thorsen et al., *Science* 298, 580 (**2002**). © 2002, American Association for Advancement of Science.

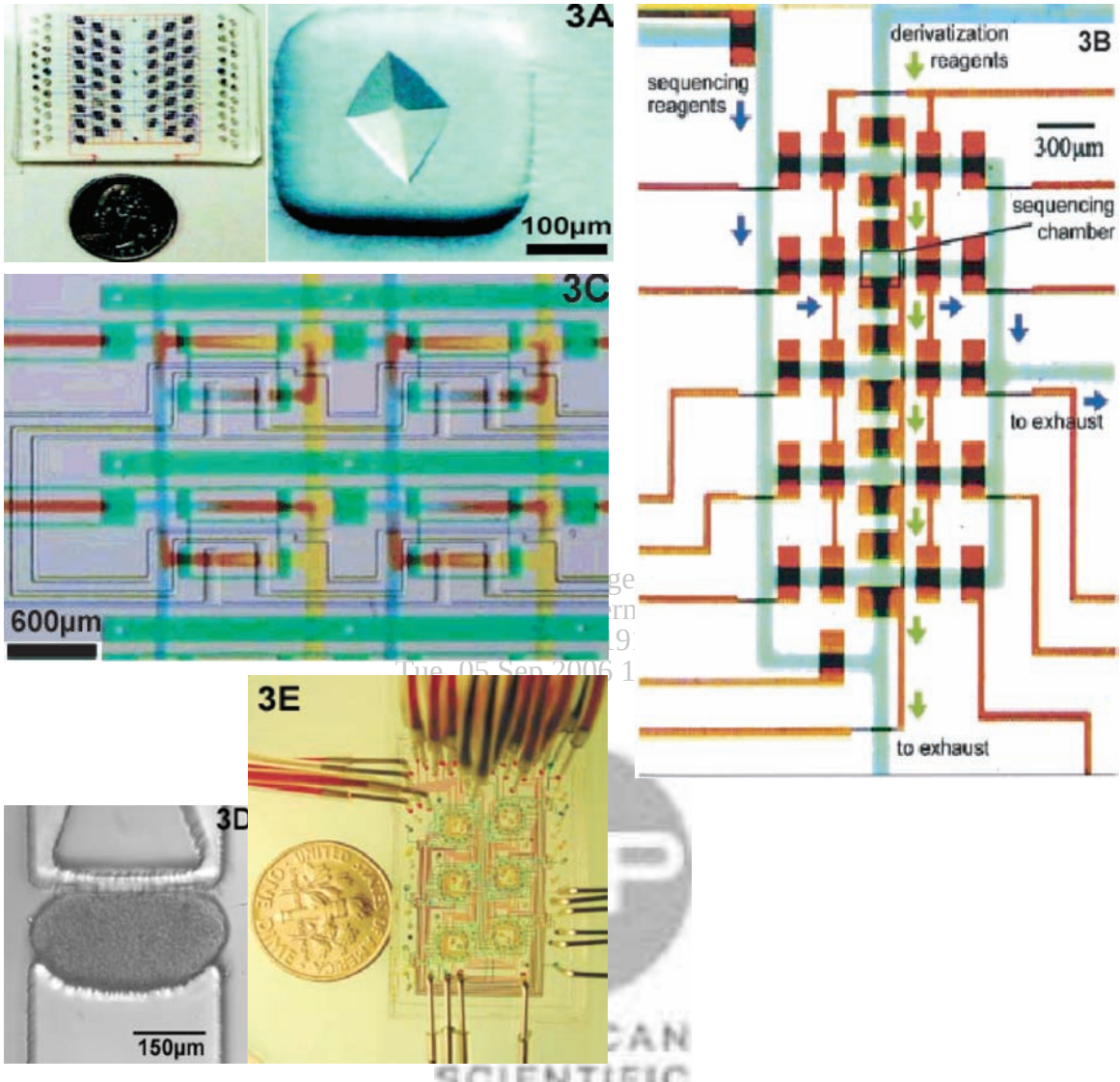

Fig. 3. *Biological applications.* The following are a few examples of microfluidics in biology: (A) Protein crystallizer. Reprinted with permission from [5], C. L. Hansen et al., *Proc. Natl. Acad. Sci. USA* 99, 16531 (**2002**). © 2002; (B) DNA sequencer-by-synthesis. Reprinted with permission from [6], E. P. Kartalov and S. R. Quake, *Nuc. Acids Res.* 32, 2873 (**2004**). © 2004; (C) PCR machines. Reprinted with permission from [8], J. Liu et al., *Anal. Chem.* 75, 4718 (**2003**). © 2003; (D) Pancreatic islet trapper. Reprinted with permission from [21], J. V. Rocheleau et al., *Proc. Natl. Acad. Sci. USA* 101, 12899 (**2004**). © 2004; (E) Bioreactor. Reprinted with permission from [23], F. K. Balagadde et al., *Science* 309, 137 (**2005**). © 2005.

we must relax the self-imposed restriction of homogeneity. So, we allow different species to be present in the same device at the same time, but we still restrict them from mixing with one another. For purposes of patterning, this relaxation enables multiple species deposited in parallel by a single device.

If we lift a further restriction that only one device be used, then it becomes possible to pattern using one device, peel it off, replace it with another, and pattern again. The characteristics of this technique coincide with the requirements of an arrayed immunoassay. The first device lays down parallel lanes of monoclonal capture antibodies onto the substrate. Then the second device transports samples along parallel channels in a direction orthogonal to the initial patterning. As a result, a 2D matrix of capture lanes versus samples is produced. Then the second device is peeled off and the ELISA-like stack is completed with polyclonal antibodies as desired. Thus simple channels, surface patterning, and switching devices on the same substrate combine to produce immunoassay arrays. As a chip-enabled technology, immunoassay arrays can then be used in related applications e.g., in biosensing.[26–30] The same ideas can be used to produce patterning within PDMS channels, e.g., in cell capture.[31]

2.1.2. Miscible Species

Up to this point, there has been an implicit self-imposed dogma that two or more chemical species are not allowed to mix inside the channels of the same device. This rule

makes sense in any application where such mixing is undesirable, but what if mixing is allowed?

If two parallel channels are connected by a far smaller channel of high fluidic resistance, then a step function in conditions has been established. This capability makes one think of chemotaxis and neuronal growth as both are directional and governed by chemical gradients. Then a simple system for cell interrogation results if a neuron is captured at the small junction between the channels and conditions are varied in the two channels to promote axonal growth across the high-resistance connection.[20]

For chemotaxis, ideally we would like to have a smooth linear gradient over the width of the experimental area so as to see how far a cell would propagate and thus measure the chemical species concentration most favorable to the cell. Gradients are produced when samples of different concentrations are combined. So, let us consider the combinatorial problem of mixing samples.

The smallest number of such constituents is two and the simplest combination is a blank (e.g., a buffer) and a non-blank (e.g., a chemical species inside the same type of buffer). If the two mix, the only change is in the concentration of the non-blank. To control that concentration, we must control the conditions of the mixing as dictated by the laws of hydrodynamics.

The small channel dimensions and low flow velocities in PDMS microfluidic devices result in low Reynolds numbers and laminar flow. The throughput, or volume flown per unit time, V, is given by Poiseuille's law, $V = \pi * \Delta P * A^3 * B/(8 * \eta * L)$, where ΔP is the pressure difference at the ends of the pipe, A is the smaller lateral dimension, B is the larger lateral dimension, η is the dynamic viscosity of the fluid, and L is the length of the pipe. In PDMS microfluidics, the width of the channel, W, is typically far larger than its height, H, and thus Poiseuille's law becomes $V = \pi * \Delta P * H^3 * W/(8 * \eta * L)$. This equation gives a very good approximation for the observed throughput although other phenomena may also have some effect, e.g., fluid flow through capillary action and/or water evaporation through the polymeric matrix.[32]

Poiseuille's law tells us that if the same pressure is applied to two channels of identical dimensions filled with fluids of the same viscosity at species concentration of 0 and 1 and joining into a Y-shape, the resulting equal throughput rates would generate a concentration of 0.5 in the joint channel. In essence, we get the mean value of the two starting concentrations.

What if now this resulting 0.5 concentration is mixed in the same way with a blank, or a pure non-blank? Clearly, the resulting concentrations would be 0.25 and 0.75, respectively. Then the profile becomes {0, 0.25, 0.5, 0.75, 1}. If the same is repeated between adjacent channels, further smoothing will result, the asymptote being a straight line from 0 to 1. Such a device would produce a linear gradient in concentration, which can be captured by a homogeneous surface chemistry, producing the final device for chemotaxis.[33] While the simultaneous ongoing diffusion would produce deviations from the predicted profile, those can be minimized in the particular devices by limiting the available diffusion time through faster flow, shorter travel distances, etc.

To go one step further, what if the architecture is the same as the above *gradient maker*[33] but the input pressures are different? Then the resultant concentration would change accordingly and the gradient would no longer be linear but would be determined by the ratio of pressures.

Although it is more flexible, controlling pressure is not as convenient nor is it as elegant as tuning by channel dimensions. To skew the mixing ratios while keeping input pressures equal, it would be enough to increase the dimensions of the preferred channel by an appropriate factor. Since throughput increases linearly with the wider channel dimension, if the non-blank channel in a mixing stage is narrower than the blank channel by a factor of (F-1) then the resultant concentration in the output channel will be $1/F$. After many stages of mixing, the asymptotic case would produce a continuous but non-linear gradient. Such a patterned concentration profile is useful in cases where the wider dynamic range is more desirable than the linearity of the gradient.[34]

What if the same bi-tone mixing is done under different rules? For example, what if we start with concentration 1 and then mix each result with a volume of blank? To produce a dilution factor F, the mixing ratios would be 1: (F-1) with corresponding proportions in the channel dimensions, e.g., keeping L and H the same but increasing one of the W's by a factor of (F-1). Such a *serial diluter* with $F = 2$ requires only 10 stages to produce a dynamic range of 10^3, while it is clear that wider ranges are possible with greater F and number of stages.[12]

Both the gradient maker and the serial diluter would benefit from a method that would allow better mixing inside the channel. The low Reynolds numbers of the discussed microfluidic devices make laminar flow dominate the scene. The general lack of turbulence makes mixing more difficult with respect to the macro world, although diffusion is faster at these smaller dimensions ($t \sim L^2$). A way to produce turbulence is to break the symmetry of the channel surface, e.g., by grooves.[35, 36] The result is a passive mixing device that produces vortices along the channel—*a chaotic mixer*.

2.1.3. Immiscible Species

The diluter and gradient maker mixing schemes discussed above are based on the miscibility of the two components. This observation naturally brings up the question "What happens if the two species are immiscible?" Water droplets in mineral oil can be reproducibly formed with characteristics programmed by conditions.[37] The produced *vesicles* can be used to compartmentalize and transport cells and reagents, e.g., for serial experiments with continually

varying parameters. Alternatively, air bubbles introduced in a long dead-end channel can serve as an inbuilt *pneumatic spring* to allow back-and-forth oscillations of compartmentalized samples over the same surface derivatization targets, e.g., in DNA microarrays. Interestingly, the pneumatic spring was shown in PMMA,[38] but there is no reason why the same would not work in denser PDMS.

2.1.4. Non-Newtonian Fluids

If we use non-Newtonian fluids and allow the channel dimensions to vary while the channels themselves remain in a single plane, then interesting effects result from the interplay of pressure, viscosity, and fluid element extension rate, such as *fluidic memory and logic*[39] and a *rectifier*.[40] These phenomena may help produce control devices in the future and might be influential in the microfluidic transport of concentrated protein solutions.

2.1.5. Varying Vertical Dimensions

In the discussion above, channel widths were varied while channel height is uniform across the device. What if the channel height is allowed to vary? Then there would be vertical bottlenecks similar to the horizontal ones above. What advantages are bestowed by the vertical bottlenecks? In soft lithography, horizontal dimensions are controlled by the mask, whereas vertical dimensions are controlled by the photoresist and the spin speed. Height can be spun precisely within as little as a micron by use of the appropriate photoresist, whereas transparency masking is practically limited to perhaps 20 microns by the technology of the laserjet printer. This means that vertical fabrication control is generally more sensitive albeit within a smaller dynamic range.

To make a vertical bottleneck in the channel, two layers of photoresist must be spun separately at different heights.[13] Then an object of size between the heights of the lower and taller channels e.g., a mammalian cell (12–30 μm) or microbead, will be stopped by the resulting *partial septum* or simply stopped at the mouth of a smaller channel. Once a cell is trapped, it can be interrogated by chemical[21] or electrical means.[22] If a partial septum is fabricated next to the mouth of a smaller channel, the flap is pushed open by flow in one direction and slammed shut by flow in the opposite direction, thereby acting as a microfluidic *diode*.[41]

A bead trapped by a partial septum would not be washed away down the channel when new reagents are fed in. Then the bead can serve as a derivatization surface for multistep reactions, e.g., for immunoassays,[13–18] with the added convenience that the capture chemistry is already present on commercially available beads and thus does not need to be built *in situ*. Furthermore, if one bead can be trapped, then so can a multitude of them, forming an *affinity microcolumn* inside the channel.[13]

To recapitulate, the realm of 2D devices has produced a surprising richness of components, including simple channels, surface patterning, a gradient maker, a serial diluter, a chaotic mixer, a vesicle maker, a pneumatic spring, a rectifier, and a partial septum. However, these devices are limited to continuous steady state operation or are simply used to fabricate other devices. In a sense, the latter are auxiliaries, while the former are like a house with all faucets running simultaneously all the time. What makes the plumbing of a house workable is the ability to stop and resume flow at different junctions at arbitrary times as desired. This basic necessity brings us to development of multilayer devices, microfluidic valves, and the components they enable.

2.2. Multilayer Devices

The previously discussed devices had the limitation that the microfluidic channels were all fabricated in a single PDMS layer. If that restriction is lifted, the resulting stacks of channels can have new functionalities.

Combinatorially, there are three ways in which two layers can be assembled: with their channels facing one another (Fig. 4A), facing away from one another (Fig. 4B), or facing the same direction (Fig. 4C).

2.2.1. Face-to-Face

We already have considered an example of the first case: The chaotic mixer. Since its grooved substrate is made of PDMS,[36] the mixer can be viewed as a single-layer or a two-layer device from the viewpoints of function or anatomy, respectively. Apart from that notable exception, (currently) nothing seems to be gained by using a face-to-face multilayer device instead a single-layer device.

What if the channels in the two layers are separated with an impermeable slab? That would simply produce

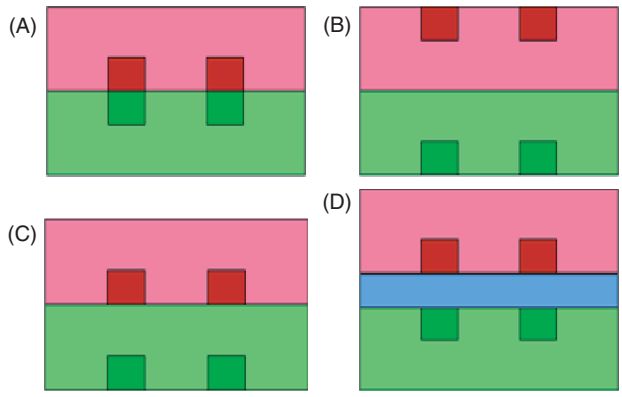

Fig. 4. *Multilayer device combinatorics.* Device layers can be arranged with their channels facing one another (A), facing away from one another (B), or facing the same direction (C). The first option has enabled chaotic mixers,[36] while the third is the basis for microfluidic valves,[46] and all their consequent devices. The first option when combined with an interjecting porous substrate (D) has led to 3D devices[42] and integrated microfilters.[44]

two single-layer devices that happen to share a common substrate. Clearly, that substrate must be somehow modified to allow for extra complexity, and therefore hopefully extra functionality. In a sense, this structure is not unlike early semiconductor transistors, which looked like little more than two diodes slapped together face-to-face as they shared a common half. In the same way as the field effect produced a new electrical functionality, permeability of the common substrate here should be expected to produce a new microfluidic functionality (Fig. 4D).

Now if the slab would have vertical channels fabricated in it that fluidically connect the channels of the two PDMS layers, then the emerging devices boast three-dimensional channels.[42] These devices allow two channels fabricated in the same layer to cross without mixing by simply having one of the channels take a vertical detour until the other channel is negotiated. This capability enables more complex patterning schemes, e.g., for proteins and cells,[43] as well as another way to make immunoassay protein arrays.[15] In two of these devices, the slab is a PDMS membrane,[42, 43] while in the third it is a silicon wafer.[15]

In the above examples, the vertical channels were on the same scale as the width of the horizontal channels they connected. What if the slab's openings are made much smaller instead? Then cells or even very large molecules can be limited to one side, while the rest of the material is let through. In that case, we have an in-built *microfilter*[44] that can be used to purify samples before the next functional stage. In the particular case, the slab is made out of polycarbonate, but one can envision different filtering materials, e.g., etched-through silicon wafers or anodized aluminum filters.

The slab can be further sophisticated by containing silicon devices[45] and possibly MEMS to add further functionality. A powerful advantage of this integration approach is the orthogonality of properties and functions inherent in the silicon and silicon components. That orthogonality allows any new advance in either area to produce immediately compatible components for the integrated systems. We shall revisit this topic further below.

2.2.2. Back-to-Back

In the back-to-back configuration, the channels share a common slab, whose thickness seems to preclude any interesting interaction (Fig. 4B). Perhaps herein lie useful devices that have yet to be discovered.

2.2.3. Face-to-Back

In the final configuration, the two layers are assembled with the channel sides pointing in the same direction (Fig. 4C). Then the channels in different layers end up separated by a membrane forming the roof of the lower channel and the floor of the upper channel at the intersection of the two channels. If the membrane is stiff enough, the resulting device is functionally the same as two single-layer devices. However, what if the membrane is not stiff enough? Then pressure in the upper channel will depress the membrane and pinch off the lower channel, much like a foot stepping on a garden hose. The resulting device is a microfluidic *pneumatic valve*.[46]

Now that we have a new device, we can characterize its properties. What makes a valve functional is the combination of its fabrication parameters and the operating pressures. At the same thickness of the membrane, larger valves have a smaller spring constant but experience larger forces at the same pressure. These two factors combine to make the closing pressure increase faster than linear with increasing valve dimension.[47]

If we view that dependence by fixing dimensions and varying pressures, we derive the basis of valve control—applying high pressure closes the valve, while applying low or no pressure leaves the valve open. The process of closing however is not truly discontinuous, i.e., there is an interval of applied pressure in which the valve is partially closed. If the pressure is precisely controlled, this intermediate state can be used to allow for a three-state valve,[19] which can be used for example as a bottleneck to trap cells.

Conversely, if we vary the dimensions but fix the pressure, some valves will be closed and others will remain open. But, morphologically, valves are just channel crossings. Is there an advantage to leaving smaller channel crossings open at the applied pressure? The answer is yes, if what we want to do is for the control channel to convey the pressure across a flow channel without a valving effect. This combination of fabricational and operational parameters produces a *crossover*. The crossover is a very useful and commonly used component due to the generally two-dimensional nature of channels in devices fabricated by multilayer soft lithography.[46]

The architecture of the original valves[46] is such that the control (pressure) channels are above the flow (reagent) channels. Since pressure closes the valves by depressing the membrane, these components are called *pushdown*. Inverting the layer stacking would produce other valves that close by deflecting the membrane upward;[47] accordingly such are called *pushup*.

Pushdown valves generally require higher closing pressures than pushup valves for the same dimensions because they need to overcome the arch effect in the membrane while the pushup valves do not. This advantage allows pushup devices to valve deeper channels, which are required e.g., in working with mammalian cells. The same can be done with pushdown devices but at the expense of significantly larger valve dimensions and higher closing pressures. On the other hand, unlike pushup devices, pushdown devices allow flow channel access to the substrate. This feature is critical in applications such as microarrays where the DNA or proteins are printed on a glass slide before it is assembled to the microfluidic chip.

2.2.4. Combinations of Components

Having characterized individual valves, we can move one level up in the anatomical hierarchy and ask what kind of combinations can be produced among multiple valves and channels. If multiple valves are connected to the same control channel and close at the same pressure, we get a *valve array*[2] (Fig. 5A). The valve array is an invaluable component that drastically simplifies the control layout of the chip and thus enables incredible density of parallel-control subdevices within the same chip.

Combinatorially, a valve array is multiple valves connected to the *same* control channel and controlling *separate* reagent channels. The italicized variable states generate four permutations: {same, separate}, {separate, separate}, {same, same}, and {separate, same} (Fig. 5).

The case {separate, separate} is multiple valves controlling multiple channels independently, which describes the generic case and thus is of no special interest (Fig. 5B).

In the {same, same} case, if the valves are connected to the pressure source much like the teeth of a comb and with *negligible* fluidic resistance between consecutive valves, then all valves close simultaneously and break up the reagent channel into compartments, or *chambers*[2] (Fig. 5C). Such compartmentalization is useful when multiple identical experiments are to be done in parallel, e.g., to test reproducibility and extract better uncertainties.

What if in the {same, same} case, we instead build *significant* fluidic resistance between consecutive valves? Poiseuille's law suggests that this can be done with long narrow connecting channels. Then liquid would preferentially fill up the pocket of a valve and thus close it, instead of traveling through the high resistance connector to the next valve. So, the valves would close one after the other instead of closing simultaneously as in a valve array. That would still compartmentalize the reagent channel, but it would also produce a net transfer of volume in the forward direction, because each closing valve displaces a volume while its already closed predecessor prevents backward displacement.

To return this device to the original state, pressure is released at the pressure source, so the valve having closed first will open first. Since all other valves are still closed, this negative displacement pulls reagent liquid into the same direction as before. The same happens with the next valve, and so on. Thus in both the closing and opening strokes, this device displaces reagent liquid in the same direction, thereby acting as a one-directional pump. The direction of pumping is always away from the pressure source and thus is hardwired during fabrication.[7] The high-resistance connectors give the device a distinctly snake-like appearance, so it is natural to dub it a *serpentine pump*. The biological equivalent of the serpentine pump is the peristaltic movement inside our own gastrointestinal tract where sphincter muscles imbedded in the walls contract in sequence to transport matter along.

The {separate, same} case, i.e., multiple valves connected to separate control lines and controlling the same channel, seems redundant at first (Fig. 5D). Compartmentalization still holds, but the design is certainly inefficient in parallel-processing applications. Multiple controls allow for the valves to be opened and closed separately. If we mimic the serpentine pump sequence, there will clearly be a reliable pumping in one direction, provided at least three valves are used. This sufficient condition created the colloquial name for such components, *triplet pumps*.

If we compare the architecture of a serpentine and a triplet, the striking result is that a serpentine is asymmetric, whereas the triplet is completely symmetric. The asymmetry of the serpentine hardwires its pumping direction, so we must expect that the symmetry of the triplet would allow pumping in either direction. This is indeed so and is accomplished by simply reversing the closing sequence.[46]

Both types of pumps have advantages and disadvantages. Serpentines require only a single control channel but can pump in only one direction hardwired during fabrication. To allow pumping in either direction, a set of two serpentines with opposite chirality can be fabricated, but then the number of control channels is increased to two, while more space is required on the device. Serpentines also lose efficiency with small turn lengths because the retardation between successive valves is not large enough to ensure complete closing/opening of each valve before its successor starts closing/opening. Triplets can pump in either direction and can work within smaller real estate, but they do require three control channels instead of one or two. Overall, the triplets find wider usage because the serpentines require significantly larger space to function efficiently.

Incidentally, the middle valve in the triplet pump can be replaced by a disproportionately bigger valve, which can serve as a micropipette[19] of known volume or volume controlled by applied pressure.

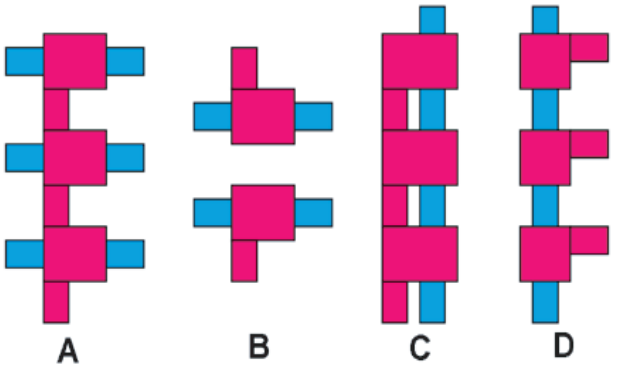

Fig. 5. *Valves and channels combinatorics.* Valves (red) and the channels they control (blue) can be arranged in four ways depending on mutual connectivity to produce: (A) Valve arrays;[2] (B) The generic case; (C) Compartmentalization[2] and serpentine pumps;[7] (D) Triplet pumps.[46]

Now that we have completed the four possibilities, what if we parallelize each of them either with respect to flow channel or control channel?

2.2.5. Parallelization

The parallelization of {same, same} produces nothing new other than further compartmentalization if applied to valves, or economy of control lines if applied to flow channels. The parallelization of {separate, separate} is just a more populous version of the same generic case. The parallelization of {same, separate} when applied to flow channels produces economy of control lines. The parallelization of {separate, same} when applied to valves produces more compartmentalization. Thus far, nothing exciting is observed.

Curiously, the parallelization of {same, separate} when applied to valves is equivalent to the parallelization of {separate, same} when applied to flow channels. While both seem just a special case of the generic {separate, separate}, it is an interesting case nonetheless. In essence, it is a matrix of crisscrossing channels that make valves at the intersections. If these valves are all of the same size, then functionality is not very exciting as any of the control channels would close all flow channels. But, what if the valve size is allowed to vary, e.g., some are true valves and others are crossovers? Then each control line can only close a hardwired subset of the flow channels. This condition brings about an analogy with binary logic in electrical engineering to produce a microfluidic *multiplexor*.[2] The binary multiplexor logic allows N flow channels to be controlled by $2 * \log_2 N$ control channels, as long as the restriction holds that only one flow channel is open at a time. This means each doubling of the number of controlled channels requires only two more control channels. Furthermore, it is possible to construct multiplexors controlling multiplexors using a combination of pushup and pushdown devices.[2] Thus the multiplexor is a primary enabler in large-scale microfluidic integration.[2]

2.3. Surface Modification

The surface of PDMS is relatively inert and hydrophobic due to the $[-(CH_3)_2SiO-]_n$ backbone of the polymer. The chemical and biological inertness is generally a desirable feature as it allows for minimal coupling between the species transported and the microchannels guiding that transport.

However, the hydrophobicity of the surface decreases the wetting angle with water and thus increases the capillary resistance experienced when water is being forced into the channels. In addition, hydrophobic chemical species are more likely to attach to the surface non-specifically,[48,49] thereby potentially producing a noise problem, e.g., in fluorescence studies. Accordingly, a number of techniques are used to make the PDMS surface hydrophilic by oxidation,[50] including treatment with oxygen plasma,[51,52] corona discharges,[53] a combination of UV light and ozone,[54] and covalent grafting of highly polar molecules, e.g., polyethylene glycol.[6] Alternatively, sacrificial blockers are used, e.g., BSA (bovine serum albumin), to saturate the exposed channel surface and thus prevent further attachment of the important species.[16]

On the other hand, the functional chemical inertness of PDMS makes it difficult to produce binding strategies for situations where it would be beneficial to be able to trap the species of interest in the channel. Anchoring the molecule or cell to the PDMS surface would allow the species to be subjected to subsequent consecutive interactions with different reagents. Examples of such applications are: DNA sequencing-by-synthesis,[6] where the same DNA molecules must undergo consecutive base-extension reactions with different nucleotides; general immunoassays, where the protein stack is built by consecutive exposures to different proteins;[16,17] and *in-situ* oligonucleotide synthesis where the immobilized strand grows by consecutive exposures to different monomers.[55]

One way to accomplish such a desirable surface deposition is for the afore-mentioned non-specific binding to be utilized as a solution rather than viewed as a problem.[16,56,57] In that case, the species of interest are purposefully allowed to bind non-specifically to the channel wall or substrate. However, any remaining active sites may need to be passivated with blockers[16] so that no further non-specific attachment happens in the later stages of the experimental procedure.

Another way to produce deposition is covalent bonding of vinyl groups to –SiH moieties on the PDMS surface.[6] That is a widely applicable method due to the commonality of vinyl moieties in a large number of chemical species that thusly become bondable to PDMS. As in all specific surface chemistries, the presentation of one standard functional group allows the grafting of a desired species through a series of well-known reactions involving commercially available linkers.

Finally, there are cases where none of the above strategies, or a combination thereof, is sufficient to prevent undesirable attachment. In that case, an active method of accretion removal is available through microfluidic means. In the particular case of cells growing in a bioreactor,[23] cells tend to attach to the PDMS channels over time and eventually hinder operation of the device. So, the reactor is divided into sections to be cleaned periodically on a rotating basis by lysing agents. Since only a small portion of the reactor is sanitized each time, the overall population of cells does not suffer significant loses. The same idea is clearly applicable where the accretion is a chemical species, with the obvious caveat that the cleaning agent must be chosen accordingly.

2.4. Heterogeneous Integrated Devices

Up to this point, we have exclusively considered components fabricated in or from PDMS (channels, valves, and

combinations thereof) combined with a *passive* substrate to form the overall device. Relaxing the PDMS and *passive* restrictions allows heterogeneous devices where components may be fabricated in different materials contributing different properties.

We already considered integrated polycarbonate microfilters[44] as well as bead-based affinity microcolumns,[13] but other possibilities abound. For example, if electrical devices are fabricated in the substrate of a PDMS chip, new combinations of functionalities become possible, e.g., a capacitance cytometer,[10] a thermal cycler,[7] and actuating valves using Braille pins.[57] Moreover, such devices can be arranged as independent but interconnected modules functioning within the bounds of a single chip.[45] The same ideas can be pursued with integrated optical devices.[58]

Generally, integration of non-PDMS devices with PDMS microfluidic chips is still in its infancy and we can expect important results from this part of the field in the future.

3. BIOLOGICAL APPLICATIONS

3.1. Physical Scales

As seen in Figure 6, the characteristic scales of PDMS microfluidics impose an upper limit on the biological systems to be studied in such devices. That limit is set by the size of the channels, the overall internal volume of the device, and the density of cells that can be realistically achieved and maintained in such dimensions. The typical total internal volume of the channels of a microfluidic chip is ~100 nL. The volume of a typical mammalian cell is ~10 pL. Thus 10,000 cells is the ultimate upper limit for chips of typical size, but space must be allowed for the surrounding medium and for meaningful control. Thus, unless we are talking about a continuous flow-through mode of operation such as a cell-sorter, the practical upper limit seems to be a few thousand cells in the typical chip at a time.

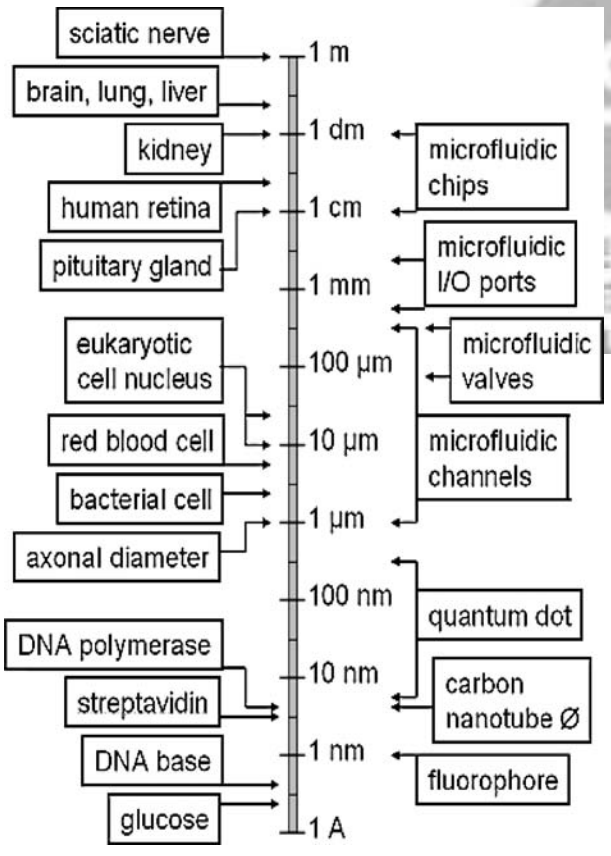

Fig. 6. *Biological and microfluidic scales.* Microfluidic devices are well suited for cellular biology and can also be applicable to molecular biology, e.g., by use of surface chemistry.

Is there a corresponding lower limit? It is reasonable to define the primary function of a microchannel as conducting flow along its length while restricting flow otherwise. Then an immediate lower bound is set by the gas permeability of the polymer matrix of PDMS. In biology however, most of the interesting phenomena occur in a water environment by/to/with cells, organelles, proteins, and/or nucleic acids. As such, the gas permeability is mostly an advantage that allows proper aeration (e.g., for maintaining oxygen-breathing cells), rather than a true restriction, unless the gas exchange is the phenomenon of interest.

Another lower limit can be set by the level of leakage of dynamic components in PDMS, e.g., valves. However, properly designed, fabricated, and operated valves have not been reported to leak measurably and so, such a limit cannot be stipulated at present.

Finally, a lower limit may be set by the size of the smallest fabricated bottleneck, as such can be used to limit the passage of a sufficiently big object, e.g., a cell, a cell fragment, or a very large molecule. The smallest channel fabricated up to this point is ~1 square micron in cross-section and as such, can stop a bacterial cell, a cell nucleus, or a very long piece of DNA. However, the aforementioned capability to employ surface chemistry on PDMS for specifically binding species of interest makes such a limit hardly meaningful. In addition, certain molecular biology applications only include a single combinatorial step and thus do not require trapping under changing conditions.[2]

To recapitulate, in terms of scale and handling capabilities, PDMS microfluidic devices are well suited for cellular biology and are also usable in molecular biology, e.g., in conjunction with surface chemistry.

3.2. Chemical Compatibility

Other potential problems to consider are cell toxicity and protein denaturation. However, studies have shown that different types of cells can survive and thrive in PDMS channels, e.g., attached to fibronectin coatings[56] or in the medium,[59] even over a long time.[23] Also, other applications such as DNA sequencing-by-synthesis[6] and immunoassays[13–18] have given examples of enzymes and

binding proteins retaining their functionality inside the PDMS channels. Thus while compatibility must be examined in each new application, there are no fundamental reasons for such problems.

3.3. Scope and Combinatorics

Since the subspace of biology accessible to PDMS microfluidics is cellular and molecular (Fig. 6), we can apply the same combinatorial methods as before to derive applications. Molecular biology studies the properties and interactions of proteins, nucleic acids, and other biological molecules. Cellular biology studies the properties of cells and their interactions with one another and with the environment. However, the chemical environment is determined by the molecular species present extracellularly, and thus the interaction of cells and chemical environment is equivalent to the interaction of cells and molecular species. Thus under commutative symmetry, the unique interactions are: {cell, cell}, {cell, molecule}, and {molecule, molecule}. Another variable is the number of objects of each type, i.e., single or multiple. Other variables are time, radiation, and temperature, but let's concentrate on mapping out the three largest subspaces above.

The {cell, cell} subspace spans all interactions among cells, e.g., one-with-one, one-with-many, many-with-many, and the parallelization derivatives of those. However, since cells signal to each other by secreted chemical species, this subspace is contained in the {cell, molecule} subspace, just as the latter is ultimately contained in the {molecule, molecule} subspace. Thus these combinatorial cases are not orthogonal but are categories that allow us to specify the level of generality in the particular context.

Examples for interactions involving cells are neuronal signal transduction, immunological response, mutual population control, hematopoiesis, and apoptosis, to name a few. Apoptosis in particular is a very active field due to its significance to cancer and aging, while stem cells hold the promise of regenerative medicine.

3.4. Microfluidic Capabilities and Addressed Applications

What PDMS microfluidics brings to the cellular biology table is the ability to isolate and manipulate a single cell or populations,[2, 9, 19–22, 43, 59, 60] allow subpopulations to interact in a controlled fashion,[60] expose individual cells or subpopulations to controlled chemical[2, 19–21, 59, 61] or electrical[22] conditions, allow for observation of the effect by external or integrated devices through optical[2, 9, 19–21, 59] or electrical means,[10, 22] and finally provide all of the above with the capability for massive parallelization.[2, 22, 42, 60] Parallelization by itself is already a critical competitive advantage that offers parsimony of material, excellent statistics through simultaneous multiple copies of the same experiment, and a combinatorial/shotgun approach to optimizations, mappings, and discovery.[2, 5, 62]

A reclassification of the same body of work from the viewpoint of addressed cell types would produce the following: neurons,[20, 22, 60, 61] pancreatic islets,[21] tumor and capillary cells,[43] avian and sheep red blood cells,[10] mouse myeloma cells,[10] rodent fibroblasts,[10] Jurkat T cells,[19] 3T3 fibroblasts,[56] osteoblast-like MC3T3-E1,[56] human umbilical artery endothelial cells,[56] and HeLa endothelial cells,[56] and bacteria.[2, 9, 59] While this is an extensive-looking list, it is certainly dwarfed by the variety of mammalian and non-mammalian cells that are the subject of meticulous study and/or extensive use in biology. Thus the potential for innovative work in cellular biology utilizing microfluidics as a new tool remains virtually untapped.

The same fluid-handling capabilities are available to be applied to molecular species instead of cells. The only handling difference is that due to the smaller scales involved, biological molecules in general cannot be trapped using channel bottlenecks, so valving and surface chemistry become the only viable options in *mutually miscible* fluids. Surface chemistry in particular is preferable as it allows exposing the species of interest to multiple consecutive chemical conditions without washing it off and losing it downstream.[6] However, surface chemistry is generally difficult to develop and perfect, while valving alone suffices in many applications, e.g., ones that require just a single combinatorial step to be performed with massive parallelism.[2]

If the above "mutual miscibility" restriction is lifted, then compartmentalization becomes possible through the use of combinations of oil, water, and air.[37, 63–65] A major advantage of such devices is the simplicity of their architecture. Another one is their suitability to low-cost rapid-screening applications where a train of microdroplet samples is railroaded over a small detector. By comparison, two-dimensional static immobilization techniques like standard microarrays require far bulkier and/or more expensive detection systems.

From a certain point of view, manipulations in molecular biology boil down to a set of protocols that deal with synthesis, replication, extraction, purification, identification, and quantitation of DNA, RNA, and proteins. These three broad molecular groups are interconnected by transcription (DNA to RNA), translation (RNA to protein), and reverse transcription (RNA to DNA), adding more protocols to the toolset. While in principle all these protocols can be done inside microfluidic chips, the only ones already shown are: DNA synthesis;[55] DNA replication;[7, 8] DNA identification by sequencing;[6] protein identification by crystallography;[5] and protein quantitation by immunoassays.[12, 13, 16–18, 27–30] Interestingly, DNA identification by hybridization has been shown in PMMA (polymethylmethacrylate),[38] but there is no reason why exactly the same cannot be done in PDMS. Since only a few of the above molecular biology methods have been

reduced to microfluidic format, it is clear that most of the work in the field still lies ahead.

3.5. Integrated Devices

Finally, we should consider integrated devices for biological applications. Most of the completed works utilize external fluidic control and data acquisition systems, such as solenoid macrovalves, syringes, arc lamps, lasers, fluorescence microscopes, cooled CCD cameras, and optical scanners. In a sense, while the chip is fluidically miniaturized, the rest of the system remains at the benchtop scale. An obvious step then is to move towards miniaturizing the rest of the system, e.g., by integrating detectors to decrease cost and improve portability. Another line of advance is to integrate multiple types of measurements inside the same device (e.g., optical, chemical, and electrical) to provide more detailed information for the studied system and/or improve reliability of results by coincidence analysis. A few steps have already been taken in that direction,[10, 44, 58] and we will certainly see many more in the future.

4. CONCLUSIONS

In less than a decade, PDMS microfluidics has matured into a powerful sophisticated technology offering the important advantages of speed, economy of scale, capability for parallelism, architectural flexibility, and high compatibility with a variety of interrogation methods. However, applications are still young.

While important biological applications have already been demonstrated, our analysis shows a still largely untapped potential for such, as well as for further sophistication of microfluidic devices by integration of optical and electrical components for orthogonal and coincidence analysis in miniaturized portable systems with precise temperature control.

Cost, speed, parallelism, and portability are major driving forces in biological and biomedical research and medical practice where groundbreaking fundamental advances, mounting operational expenses, biological variability, and complexity demand respective revolutionary advances in handling technology and methods. Microfluidics has the capability to provide such advances and thereby make ubiquitous diagnostics and affordable personalized/preventive medicine a reality.

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