Review

Integrated microfluidic systems for high-performance genetic analysis

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Driven by the ambitious goals of genome-related research, fully integrated microfluidic systems have developed rapidly to advance biomolecular and, in particular, genetic analysis. To produce a microsystem with high performance, several key elements must be strategically chosen, including device materials, temperature control, microfluidic control, and sample/product transport integration. We review several significant examples of microfluidic integration in DNA sequencing, gene expression analysis, pathogen detection, and forensic short tandem repeat typing. The advantages of high speed, increased sensitivity, and enhanced reliability enable these integrated microsystems to address bioanalytical challenges such as single-copy DNA sequencing, single-cell gene expression analysis, pathogen detection, and forensic identification of humans in formats that enable large-scale and point-of-analysis applications.

Introduction

With the completion of the reference human genome sequence and additional individual sequences [1–3], even more ambitious goals for future genome-related research are being planned. Exploring the implications of genome variation for speciation, evolution, and disease [4,5], studying gene expression and regulation at the single-cell level [6,7], as well as improving forensic and clinical genetic analysis [8,9], are now on the horizon. Technologies that will enable these advances of genetic analysis must be fast and inexpensive, have high sensitivity, and provide flexible and robust platforms.

Automated genetic analysis has advanced significantly in the past decade through the application of robotics, but several intrinsic drawbacks are increasingly evident. First, the liquid-handling limits of robotic analytical techniques are usually in the microliter scale, which not only consumes expensive reagents, but also leads to inevitable sample dilution. For example, to analyze a single gene in a cell, we conventionally put it into a working volume of 10 μL, which results in extreme dilution down to $<10^{-18}$ M. This is problematic because the most sensitive systems for DNA detection typically require concentrations in the femtomolar to picomolar range [10]. Second, during conventional genetic analysis, samples are transferred between multiple instruments, which can cause further sample dilution and loss. For instance, in DNA capillary electrophoresis (CE) analysis, the loaded sample is typically 1–2 μL, but only ~2 nL of this sample volume is effectively injected into the capillary for separation and detection [11]. Third, contamination issues become prominent when dealing with low-copy-number or single-cell samples because contaminants can overwhelm the real target signals. Current analytical processes which have multiple open sample transfer steps make contamination inevitable [12]. Paradoxically, the final analytical systems for genetic analysis typically do not require a large amount of sample. For example, only 10^6–10^7 molecules are sufficient for CE detection. While robotics provides the macro integration of analytical processes which can address some of the problems mentioned above, a fully integrated and automatic system that operates on the nanoliter scale would enable improved performance.

Integrated Microfluidic Systems

Micro-total analysis systems have the potential to overcome all problems mentioned above due to their capability of integrating multiple analytical steps into a single microdevice at the pL–nL volume scale using microfabrication technology. The advantages provided by such a "laboratory-on-a-chip" system are recognized as high-speed, high-throughput, low reagent consumption, and reduction of instrument size [13,14]. Moreover, the limited diffusion distances and concentrated reagents achieved by carrying out reactions in pL–nL structures substantially increase the sensitivity and the speed of assays [15]. By integrating the analytical process on a single device, we can achieve efficient connections between each functional unit, so that the loss and dilution of samples is minimized. Additionally, microfluidic automation eliminates the risk of the mix-up and contamination of samples. Given all these inherent advantages, fully integrated microfluidic systems are a promising technology.

In this review we will first discuss several important considerations for the design and development of fully integrated micro total analysis systems, before highlighting significant recent advances in the areas of DNA sequencing, gene expression analysis, pathogen/infectious disease detection, and forensic short tandem repeat (STR) typing.

Development of Integrated Microdevices

To develop fully integrated microsystems for gene expression and genetic analysis, four elements critically impact process integration: i) device material, ii) heaters and temperature sensors for thermal cycling of reactions, iii) microvalves for partitioning analytical steps, and iv)

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sample/product transport between analytical steps. The choices made in each of these areas determine the challenges and successes achieved in the integrated analytical system.

Device materials

The choice of device materials is important for the development of integrated microsystems because the design, fabrication and operation of the device are heavily dependent on substrate properties. Glass remains the most extensively used substrate for implementing integrated microfluidic devices for genetic analysis due to its particular advantages, including high dielectric strength, optical transparency for detection, and mature surface chemistry manipulation [16,17]. The primary disadvantage of glass is its high material cost and more complex fabrication. However, disposable glass microchips can be made at low cost when manufactured in high volume [18]. Plastics and elastomers such as poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) have also been successfully utilized in microsystems, and are becoming increasingly popular [19,20]. In contrast to glass, the simple fabrication and low material cost make plastic and elastomers better choices for disposable devices. However, these materials have some fundamental challenges due to their lack of facile surface modification techniques, inherent fluorescence/Raman background, low glass transition temperature, and incompatibility with metal microfabrication for sensor integration.

Temperature control

Gene expression and genetic analyses usually include DNA or RNA amplification steps, which require rapid and accurate temperature control for thermal cycling of reagents in microreactors. Many temperature-control methods, including contact and non-contact heating, have been successfully demonstrated on microdevices. Contact heating methods include external heaters attached to the chip surface, such as Peltier heaters, and microfabricated thin film heaters made of Ti/Pt [21], aluminum [22], or indium tin oxide (ITO) [23]. Non-contact heating can be realized by infrared (IR) irradiation [24]. While these heating systems demonstrate similar performance in a laboratory setting, contact heating is more suitable for point-of-care applications due to its inherently small size and facile integration and operation.

Microvalves for partitioning analytical steps

In a fully integrated microfluidic system which contains several analytical steps, microvalves are essential parts for physically separating each functional unit. Microvalves can be categorized into four groups: i) active mechanical, such as electromagnetic [25], piezoelectric [26], and pneumatic valves [27,28], ii) active non-mechanical, including phase change material valves [29,30], iii) passive mechanical, such as check valves [31], and iv) passive non-mechanical microvalves, including hydrophobic [32,33] and gel valves [34]. The selection of a particular microvalve depends on the following considerations: whether it is normally in closed or open mode, its dead volume, power consumption, pressure resistance, reusability, insensitivity to particle contamination, fabrication complexity and cost. In general, active mechanical microvalves have the best performance and are the most commonly used in microsystems. However, in a given application, other simple passive valves may be more suitable. For example, in an integrated PCR-CE microdevice, the interface between the viscous separation matrix and the PCR solution can act as a barrier to restrain the PCR solution in the reactor during thermal cycling [21]. While such a passive mechanical valve is uncontrolled and not resistant to high pressure variations within the channels, it can be sufficiently reliable in many circumstances.

Sample/product transport

Integration of an entire analytical process on a single device is much more complicated than simple combination of several microfabricated units. Efficient and reproducible sample/product transport between functional units is the key to seamless integration that demonstrates the advantages of sensitivity, reproducibility, and reliability. Methods to transport samples within a device include: i) transport by active pumps [35], ii) transport by electric field [36], iii) transport vehicles, such as DNA capture and transport using magnetic beads [37,38], and iv) capture at sample destination by filters [39], capture gel [40], or solid-phase columns [41]. The pump and electric field methods are simple but can require delicate timing optimization. In particular, when volume change occurs during transport, the sample will be discarded or diluted. In contrast, the carrying and capture methods are more efficient and reliable, particularly if it is necessary to make a large change in sample volume or exchange of buffers. A good example of the challenges arising from sample transport is provided by microchip capillary electrophoresis (μCE). Nearly all the μCE systems developed so far employ the classical cross-injector to form narrow sample plugs for CE separation [14]. If excess sample is supplied by off-chip preparations, this method works well in establishing a significant amount of analyte in the cross-injection region. However, in a fully integrated microsystem such as integrated PCR-CE devices [16,21,42], this simple electrokinetic injection can be problematic because the amount of sample provided by the nL-scale device might not be sufficient to provide a good injection.

To solve this problem, researchers have developed various inline methods for sample preconcentration and injection. For example, Ueberfeld et al. developed a DNA sample loading method using carboxyl-modified magnetic beads for DNA capture in chip-based electrophoresis (Figure 1a) [37]. As an alternative, Long and coworkers utilized a solid-phase extraction column coupled with a CE separation channel for sample purification, concentration, and injection (Figure 1b) [41]. More recently, the Mathies group developed post-PCR sample capture and inline injection methods using an oligo- or streptavidin-modified capture gel, which purified and injected PCR products with near 100% efficiency (Figures 1c and 1d) [43,44]. Integrated PCR-CE microdevices with such simple inline injectors have been successfully developed, and demonstrate significantly enhanced sensitivity (10–20-fold) and reliability because of their quantitative sample transfer capability [43].

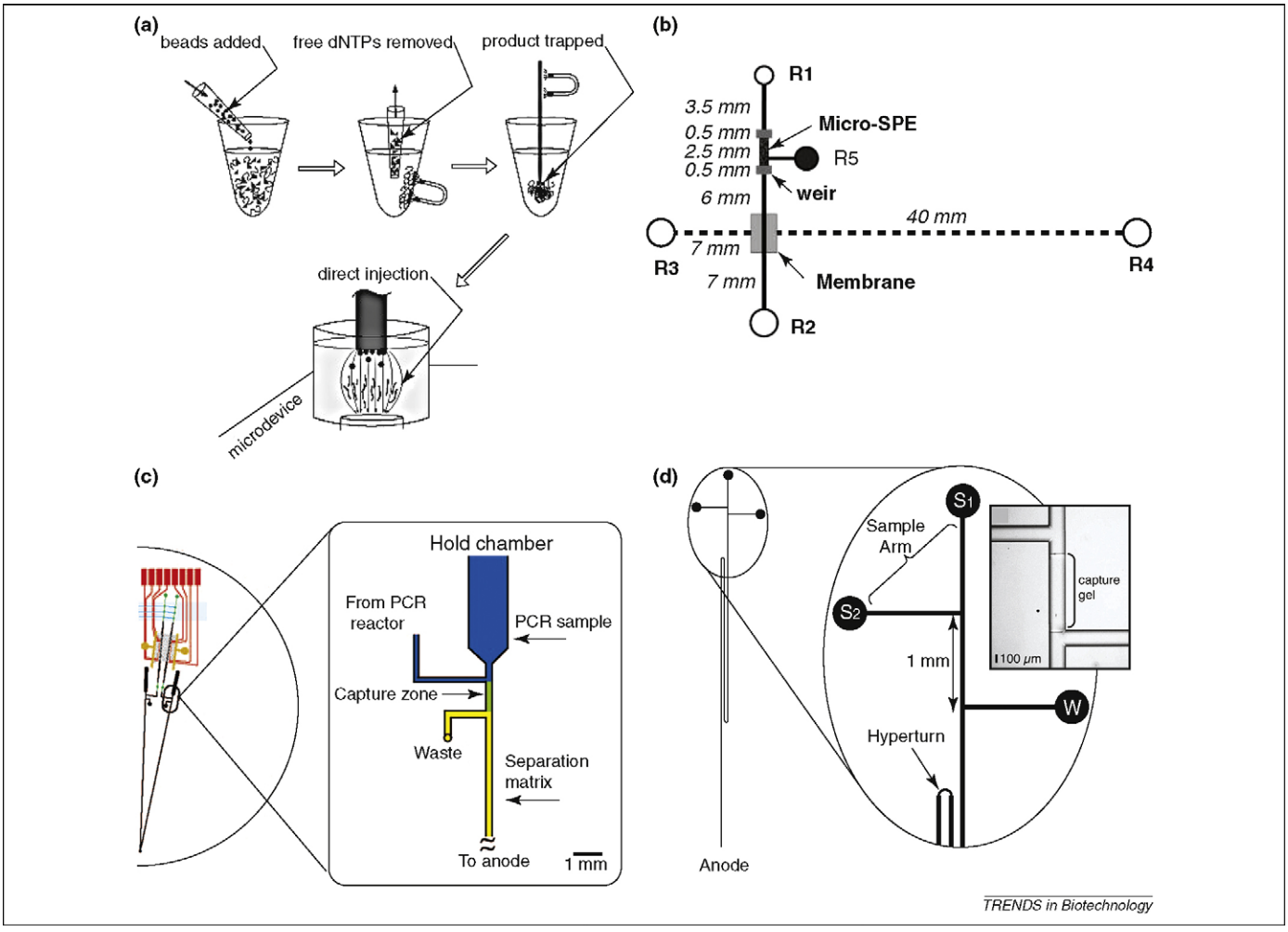

Figure 1. Methods for sample preconcentration and inline injection. **(a)** Schematic of DNA loading procedure using paramagnetic beads for chip-based electrophoresis. Paramagnetic beads are added to a tube and adsorb the DNA sample. The beads are then captured on a magnetized wire and transported to the microchip, where the wire is used as an electrode for direct electrokinetic injection of DNA (adapted with permission from Ref. [37]). **(b)** An integrated PDMS microchip with a solid-phase extraction column for sample preconcentration, injection and separation. A nanoporous membrane is sandwiched between two PDMS substrates to isolate the upper SPE channel (solid line) and the lower CE separation channel (dashed line). After sample purification in the upper channel, a voltage pulse is applied to inject the concentrated sample through the membrane to the lower channel for electrophoresis (adapted with permission from Ref. [41]). **(c)** An integrated PCR-CE microdevice with post-PCR capture and inline injection. In the enlarged schematic, a capture matrix made of 5% acrylamide/bis gel with covalently linked oligonucleotide capture probes is photopolymerized in a channel (green). PCR products generated in a reactor are injected through channels (blue) and captured on the gel plug. After washing, purified and concentrated PCR products are thermally released into a separation channel (yellow) (adapted with permission from Ref. [43]). **(d)** Schematic of a CE chip integrated with an inline injector. A 900-μm-long streptavidin-gel plug is photopolymerized next to a separation channel. Biotin-labeled PCR products are captured and injected into the separation channel for electrophoresis. The embedded photograph shows the photopolymerized capture gel in the channel (adapted with permission from Ref. [44]).

Applications of Integrated Microfluidic Devices

The development of fully integrated microfluidic devices is advancing rapidly. This development has led to significant achievements in the areas of DNA sequencing, gene expression analysis, pathogen detection, and forensic STR typing, which are discussed in detail below.

DNA sequencing

To meet the expanding demands of DNA sequencing, extensive research has been conducted to develop new sequencing techniques and to improve the Sanger sequencing method. Next-generation DNA sequencing techniques are evolving rapidly, and have been commercialized into products providing massively high throughput. For example, the 454 GS FLX instrument (Roche Applied Science) generates ~1 million reads per run at lengths of up to 400 bases (http://www.454.com/products-solutions/system-benefits.asp). Solexa technology (Illumina, http://www.illumina.com/downloads/GenomeAnalyzer_SpecSheet.pdf) and the SOLiD™ 3 system (Applied Biosystems, http://www3.appliedbiosystems.com/AB_Home/applicationstechnologies/SOLiDSystemSequencing/overviewofsolidsystem/index.htm) provide higher throughput (>300 million and 400 million reads per run) with shorter read lengths (75 bases and 50 bases). Single-molecule sequencing methods, such as the HeliScope system (Helicos, http://www.helicosbio.com /Portals/0/Documents/Helicos_SalesSpec.pdf) and sequencing detected by zero-mode waveguides (Pacific Biosciences) [45,46], have also been successfully demonstrated with performance of 400 million reads at lengths of 30 bases and 4.5 million reads at lengths of 1500 bases suggested in early reports.

Sanger sequencing providing long reads (~700 bp) with modest throughput (hundreds of thousands of reads per day) remains the best option for *de novo* sequencing of complex new genomes and low-scale applications because

of its long read lengths and flexibility in scale. There is a tremendous opportunity for improvement of Sanger biochemistry to its ultimate molecular limits and to higher throughput using microfabrication technology. Separations of sequencing samples on microfabricated CE devices have yielded impressive results [47,48], such as ultrafast separations of 600 bases in just 6.5 min on a CE chip [49], and a 768-lane sequencing system for high-throughput analyses [50]. However, the true power of a microfabricated platform lies in its ability to integrate sample preparation with electrophoresis to achieve rapid and low-cost DNA sequencing from minute amounts or even single copies of DNA template.

In 2006, Mathies' group demonstrated a nanoliter-scale microfabricated bioprocessor which integrated all three Sanger sequencing steps, thermal cycling, sample purification, and capillary electrophoresis into a 4-inch hybrid glass-PDMS wafer [17]. This fully integrated system contains a 250-nL reactor for thermal cycling, PDMS micropumps and valves for efficient sample transport, affinity-capture chambers for DNA sequencing product purification, and a 30-cm channel for CE separation. DNA sequencing from only 1 fmole of DNA template was complete in <30 min with a 556-base read-length and 99% accuracy. This breakthrough provides an excellent core engine from which further improvements can be made to explore the ultimate sensitivity limit of the Sanger biochemistry: sequencing of a single template molecule. To achieve this goal, Blazej and coworkers developed a gel-based affinity method for DNA capture, concentration, and inline injection that was integrated with on-chip CE [40]. About 30 nL of sequencing sample produced from only 100 attomole of human mitochondrial HVII template could be quantitatively immobilized in a capture gel and inline injected into a separation channel for electrophoresis. By incorporating such an inline injector into the previous nanoliter-scale bioprocessor [17], the starting template for successful DNA sequencing could be reduced tenfold, from 1 femtomol to 100 attomol: this was a fundamental advance.

A sensitivity of 100 attomol is important because this is the amount of product that could be easily produced by PCR amplification of a single template molecule [17]. This raises the question of whether it is practically possible to amplify a single template and subsequently carry out Sanger sequencing. Recently, Kumaresan et al. provided an affirmative answer to this question by demonstrating the amplification of single-copy DNA template with primer functionalized microbeads in engineered nanoliter droplets (illustrated in Figure 2a) [51]. Long-range sequencing results generated from ~100 attomol of a 624-bp product demonstrated that these amplicons are compatible with downstream attomole-scale Sanger sequencing. By integrating the technologies presented above, a high-throughput Microbead Integrated DNA Sequencing (MINDS) bioprocessor (Figure 2b and 2c), coupled with a procedure for whole genome *de-novo* sequencing (see details in Box 1) can be envisioned. The target performance of this integrated microsystem is ~50,000 bases per hour and 1 Mb/day with read lengths over 600 bases and 30 min processor cycle time. While integrating all these elementary steps into a single device is an engineering challenge, we believe it is only a matter of time before the MINDS bioprocessor is fully realized. This integrated system will probably never match the throughput of next-generation sequencing techniques, but it will provide an important platform for medium-throughput personal sequencing. It will also be uniquely suited to the *de-novo* sequencing of large and complex genomes from single templates or single cells, thereby enabling unprecedented characterization of somatic cell variation.

Box 1. The Microbead INtegrated DNA Sequencing (MINDS) method for whole genome *de-novo* sequencing.

Genomic DNA is first randomly sheared into ~2-Kb fragments and inserted into plasmid DNA using standard methods. Subsequently, millions of nanoliter volume water-in-oil droplets containing individual plasmids and primer functionalized microbeads are generated using a high-throughput microfabricated droplet generator. Thousands of these droplets are collected in a single tube and simultaneously PCR cycled in conventional PCR thermal cyclers. In the next step, the droplets are lyzed and the microbeads collected and rapidly sorted using a fluorescence activated cell sorting (FACS) machine to separate the clonal beads from the non-clonal ones. The sorted clonal beads are then introduced into the MINDS bioprocessor (shown in Figure 2), which integrates Sanger sequencing reaction, purification, inline injection, and capillary electrophoresis on a single device, for high-throughput nanoliter-scale paired end Sanger DNA sequencing. Compared with conventional whole genome Sanger sequencing, the MINDS method can achieve saving of 2–3 orders of magnitude with regard to cost, time and space by employing a nanoliter-volume integrated process.

Gene expression analysis

Even genetically identical cells with seemingly identical cell histories and environmental conditions can have significant differences in gene expression levels, due largely to the alteration of mRNA production by random fluctuations or complex molecular switches [7,52]. Additionally, many biological processes such as stem cell differentiation [53] and diseases such as cancer [54] are triggered by single-cell variation. The highly heterogeneous gene expression and microRNA levels in these cell populations are often missed by conventional techniques, which usually analyze (and therefore average) 10^3–10^6 cells. Quantitatively studying gene expression on the single-cell level is imperative to accurately understand the true mechanisms behind cellular processes. Reverse transcription PCR (RT-PCR) is valuable for single-cell gene expression analysis; by translating RT-PCR assays into microfluidic formats, significant improvements can be achieved in cell manipulation, throughput, and sensitivity ultimately to the single cell level.

For example, Quake's group constructed a multilayer PDMS microsystem that could carry out single-cell lysis, followed by affinity mRNA purification and cDNA synthesis [28]. Several chip components integrated into this microsystem assured the success of quantitative on-chip mRNA analysis: accurate fluid control achieved by on-chip microvalves and micropumps, enhanced reaction efficiency provided by nL-scale reactors, and reduced risk of sample degradation due to enclosed structures. Moreover, a key feature of this system is the utilization of oligo(dT)-modified paramagnetic beads for mRNA and cDNA capture, which provide efficient product transport without sample

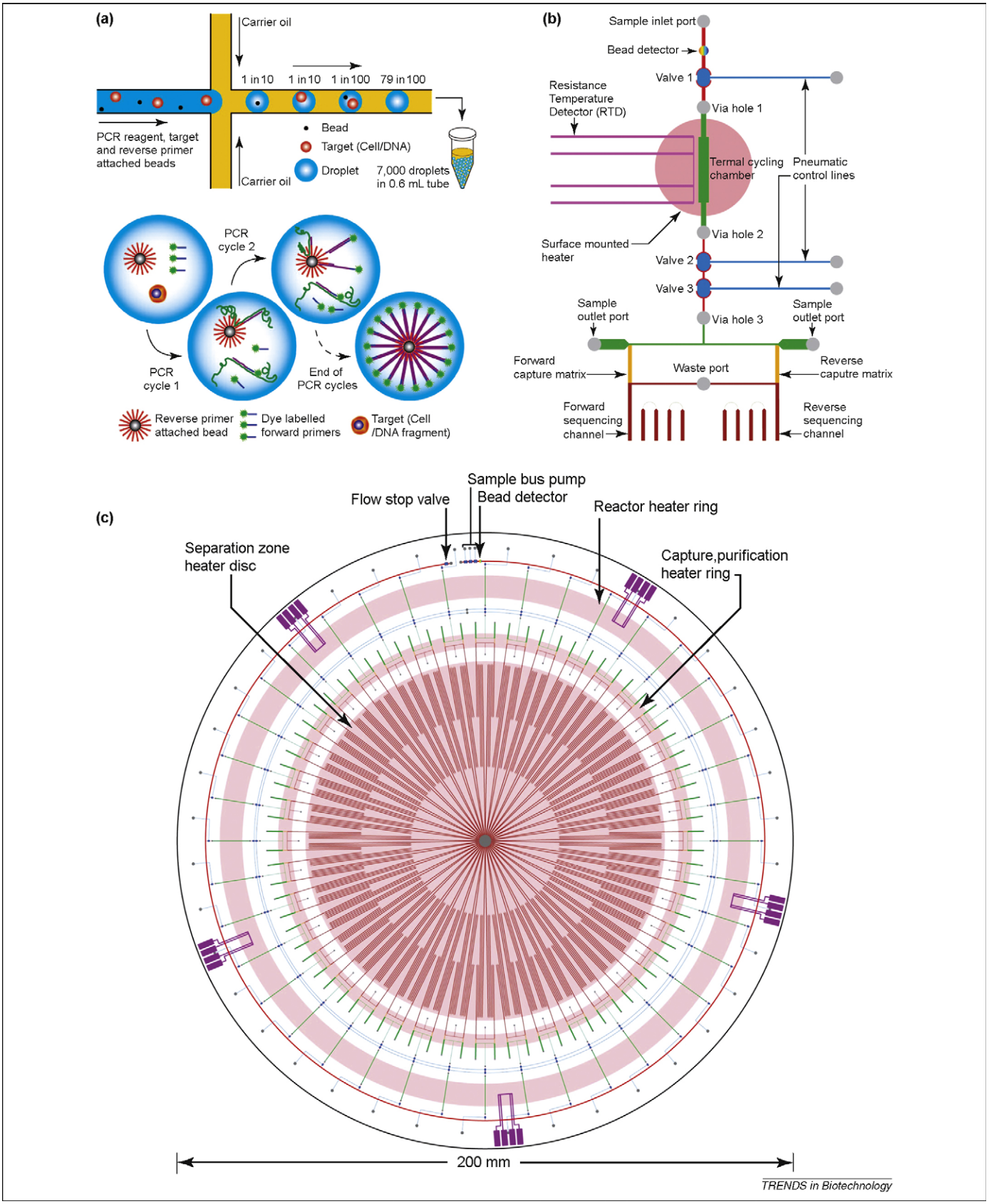

Figure 2. Integrated high-throughput nanoliter-scale bioprocessor for 100-attomole DNA sequencing generated from single templates in droplets. **(a)** Schematic of single-cell or single-copy genetic analysis (SCGA) in nanoliter droplets. Beads and template are diluted in PCR solution and pumped through a microfabricated droplet generator to form uniform PCR mix droplets. The number of droplets containing a single template copy and a single bead conforms to the Poisson distribution. Each functional droplet contains a bead modified with reverse primers, fluorescent labeled forward primers in solution, and a single template. Amplification produces many double-stranded DNA products that are linked to the bead by the reverse primer. After amplification, the beads are isolated and pooled, before being run through a flow cytometer to determine the distribution of fluorescence on each bead and to isolate possible PCR colonies (adapted with permission from Ref. [51]). **(b)** Schematic design of a double-ended sequencing chip. A diluted bead solution obtained from SCGA is pumped into the nL-scale thermal cycling chamber to place only one bead in the reactor. After

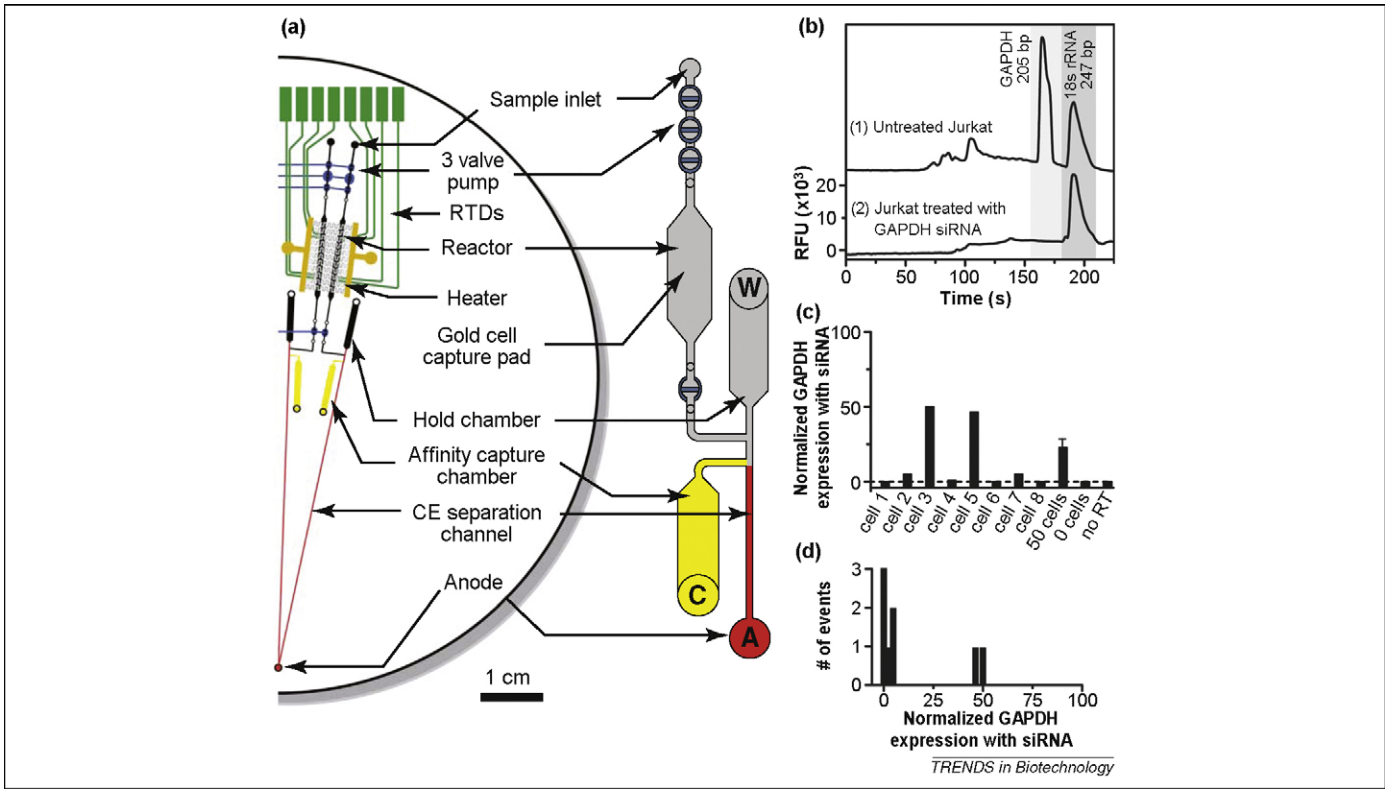

Figure 3. Integrated microdevice for gene expression analysis of single cells. **(a)** Schematic of the chip layout. This four-layer PDMS-glass hybrid device has four independent systems, each of which contains a three-valve pump (blue) for sample movement, a size-limited gold pad (gold) for cell capture, a 200-nL reactor with RTDs and a heater for thermal cycling, a hold chamber and an affinity capture chamber (yellow) for post-PCR capture and inline injection, and a CE separation channel (red). **(b)** Single-cell gene expression and silencing experiments done on the microdevice. An untreated Jurkat cell shows a 200-bp (GAPDH) and a 247-bp (18S rRNA) peak in the electropherogram, whereas a cell treated with siRNA shows only a single peak for 18S rRNA. **(c)** Gene expression of GAPDH from eight individual Jurkat cells shows GAPDH mRNA levels at 0, 5, 50, 1, 48, 0, 5, and 0%. However, GAPDH expression measured from 50 cells shows an average of 21±4%. **(d)** Histogram of the number of events for siRNA-treated cells shows two distinct populations of cells whose expression levels are very distinct from the population average (adapted with permission from Ref. [57]).

loss and dilution during buffer exchange and product collection. Using similar technologies, Zhong et al. constructed an improved system for extracting total mRNAs and synthesizing cDNA from single human embryonic stem cells [55]. Bontoux and coworkers successfully integrated single-cell trapping, total mRNA extraction, and RT-PCR into a microdevice with rotary reactors and PDMS microvalves [56]. Both systems demonstrated increased efficiency of extraction and reaction due to smaller volumes and integrated analytical processes. However, the dependence on subsequent off-chip amplification and detection limited their throughput and sensitivity.

Integrated PCR-CE microdevices developed for DNA amplification and separation should be excellent platforms for gene expression analysis using RT-PCR from single cells due to their excellent sensitivity, integrated operation, and potential for high throughput. However, until recently no such microsystem was available due to the lack of efficient sample/product transfer between each step. Toward this end, our research group recently reported a fully integrated microsystem (Figure 3) capable of carrying out single-cell capture, RT-PCR, post-PCR product capture, inline injection and CE separation [57]. The 4-inch-diameter, four-lane microdevice has several unique structures that enable quantitative and sensitive analysis: i) a gold pad in a RT-PCR reactor for single-cell capture [58]; ii) a 200-nL reactor for one-step RT-PCR in 25 min [59]; iii) a affinity gel capture structure for post-PCR purification and concentration [60]; and iv) a CE separation channel coupled with the capture structure to achieve inline injection and separation. With an estimated detection sensitivity of <11 mRNA molecules per reactor, this microsystem established the feasibility of carrying out single-cell gene expression analysis on an integrated device.

Detection of pathogens and infectious disease

Microchip technology can also play an important part in pathogen or infectious disease detection, where point-of-care analysis is highly desired. PCR-based detection technology has found the greatest use for pathogen detection because of its speed (<60 min), sensitivity (down to a single copy), as well as the capability of detecting minute amounts of targets from a huge nonpathogenic background. Many integrated PCR-CE microfluidic systems have been constructed, and demonstrated the capability of rapid, decentralized detection of various pathogens

thermal cycling, the extension fragments (forward and reverse) are pumped in parallel to two capture gels, where one lane selectively captures the forward extension fragments and the other the reverse. After capture, the chip is heated to 70 °C and the dehybridized purified band injected into the sequencing column for separation. (c) Schematic design of a 40-unit array MINDS processor for high-throughput nanoliter-scale paired end Sanger DNA sequencing on the 200-mm-diameter wafer. The processor consists of 40 thermal cycling reactors, 80 sample purification and concentration chambers, and 80 separation channels with a common central anode. A single sample bus channel along with one sample inlet port and one bead detector address all 40 microprocessors.

[27,61–63]. However, one critical drawback of these systems is that they suffer from low or even no PCR amplification when crude samples containing PCR inhibitors are processed. Integration of sample processing steps, including cell isolation and DNA purification before PCR, are required to address this problem.

To achieve a 'sample in–answer-out' capability of identifying pathogens in complex chemical or biological backgrounds, Landers's group developed an integrated microfluidic genetic analysis system (Figure 4a–c), which can carry out three major DNA processing steps: DNA extraction, PCR amplification, and electrophoretic separation [35]. The entire analysis timeline, as shown in Figure 4d, is <24 min, which is about ten-times faster than conventional methods. The successful analyses of *Bacillus anthracis* (anthrax) in 750 nL of whole blood and of *Bordetella pertussis* in 1 μL of nasal aspirate clearly indicate the potential application of this integrated system for rapid and large-scale screening of disease outbreaks.

To achieve efficient sample transfer in integrated microsystems, magnetic beads are an excellent sample transport vehicle because they can be precisely manipulated using external magnets. Beyor et al. recently developed a cell concentration and isolation microdevice using immunomagnetic beads [38]. *Escherichia coli* cells are driven through a fluidized bead bed magnetically immobilized in microchannels using an integrated on-chip pump. High capture efficiency (70%) and a detection limit of 2 cfu/μL could be obtained. More recently, this cell capture structure was integrated into a PCR-CE microdevice to achieve cell preconcentration, purification, PCR, and capillary electrophoretic analysis on a single device [64]. The magnetic beads can efficiently capture and concentrate target cells in the microchannels, and the cell-bead conjugates are precisely transported and located into the PCR chamber using on-chip pumps and external magnets, leading to an impressive sensitivity of 0.2 cfu/μL of *E. coli* O157 cells in a 50-μL input volume. O157 cells could also be selectively detected in a thousand-fold commensal background of *E. coli K12*. This cell capture PCR-CE system represents a significant advancement in the development of rapid, sensitive, and specific laboratory-on-a-chip devices for pathogen detection that address the macro-to-micro interface challenge.

Forensic STR typing

According to the US National Institute of Justice (NIJ) 2006 Annual Report (http://www.ojp.usdoj.gov/nij/about/annual-reports.htm), officials estimated a backlog of 350,000 cases of rape and homicide pending examination in the state and federal crime laboratories in the USA. In addition, compromised samples, including degraded DNA [65], low-copy-number DNA [66], and mixtures [67] are often encountered in forensic investigations and pose unique challenges to STR typing. To overcome these throughput problems, the separation and typing of STR samples amplified from off-chip PCR have been successfully done on microfabrication CE array devices, demonstrating extraordinary speed and throughput [68–70]. However, to fully appreciate the benefits provided by microfabrication technology, sample preparation steps, such as PCR and post-PCR cleanup, should ideally be integrated into the CE chips.

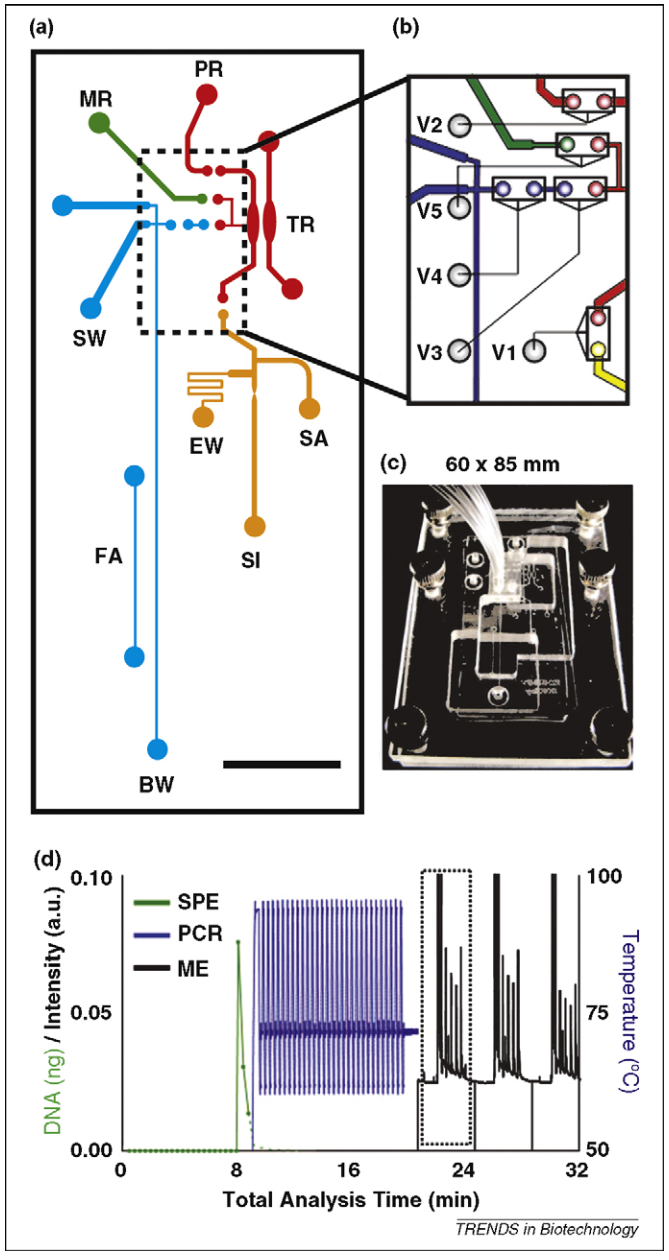

Figure 4. Fully integrated microfluidic genetic analysis system for pathogen detection. **(a)** Schematic of the microdevice. This device contains three domains for DNA extraction (yellow), PCR amplification (red), and CE separation (blue). All the reservoirs and structures are labeled as: sample inlet (SI), sidearm (SA), and extraction waste (EW) for DNA extraction; PCR reservoir (PR), marker reservoir (MR), sample waste (SW), and temperature reference (TR) chamber for PCR; buffer reservoir (BR), buffer waste (BW), and fluorescence alignment (FA) channel for electrophoresis. **(b)** Expanded view of the PDMS microvalves integrated on the chip for microfluidic control. **(c)** Photograph of the chip assembly. **(d)** The timeline of the entire analysis carried out on the microdevice. The green line is the DNA concentration released from the solid-phase extraction (SPE) column as a function of time. The blue line is the temperature cycling profile for PCR. The black line presents the three sequential traces of microchip electrophoresis (ME) (adapted with permission from Ref. [35]).

Liu et al. developed an integrated PCR-CE microdevice for forensic STR analysis, as well as a portable analysis instrument containing all the electronics and optics for chip operation and four-color fluorescence detection [71]. To explore the concept of on-site forensic human identification, we further developed an improved PCR-CE device, a 9-plex autosomal STR typing system, and a complete typing protocol (Figure 5) [72]. Real-time DNA analyses at

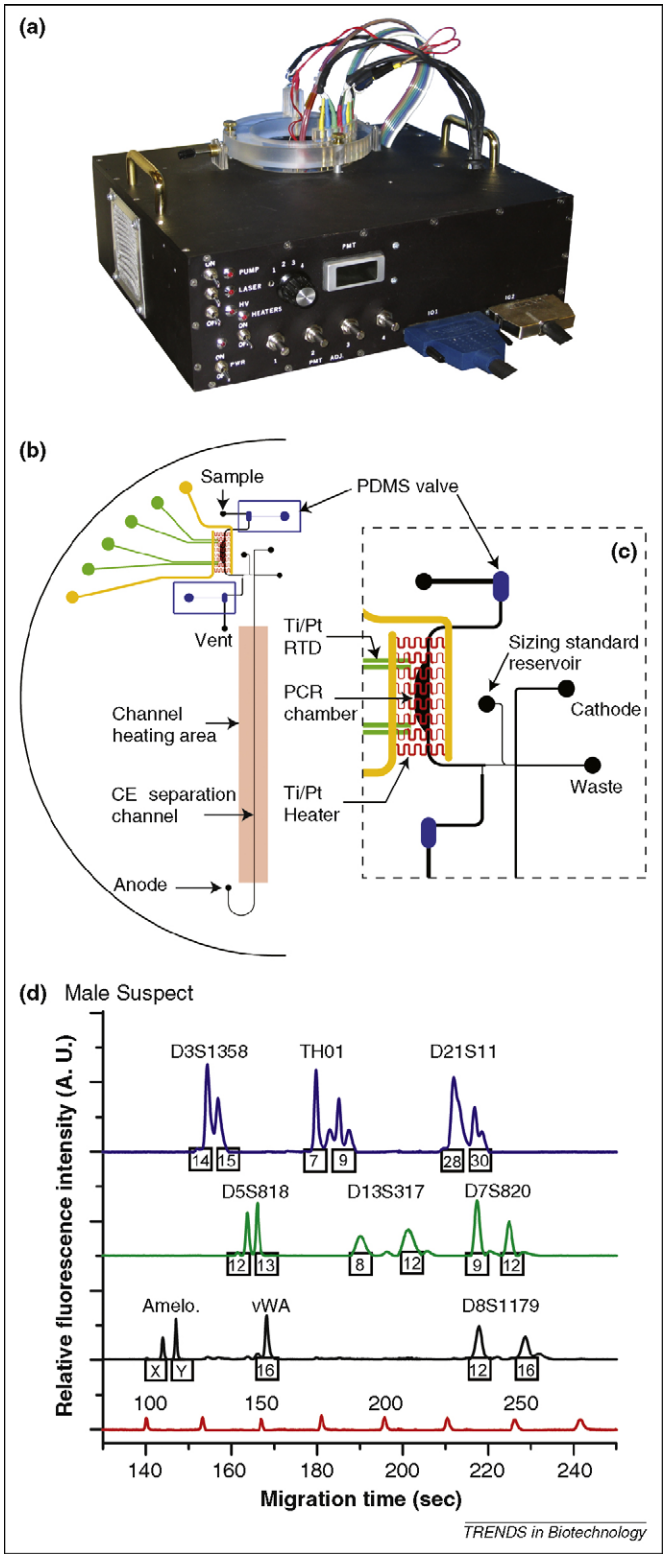

Figure 5. A portable integrated genetic analyzer for on-site forensic STR typing. **(a)** Photograph of the portable PCR-CE system. **(b)** Schematic design of the integrated PCR-CE microchip, consisting of a 160-nL PCR chamber with a microfabricated PCR heater and a temperature sensor for on-chip PCR, a co-injector for the injection of a sizing standard, and a 7-cm-long separation channel for electrophoresis. **(c)** Expanded view of the PCR and injector region. **(d)** Male suspect's 9-plex STR traces generated on-scene in Palm Beach (FL, USA) using the portable microsystem. This profile was searched against the local CODIS database and a hit was obtained successfully (adapted with permission from Ref. [72]).

a mock crime scene were carried out in collaboration with the Palm Beach County Sheriff's Office (FL, USA). A mock crime scene was investigated following standard procedures, and three bloodstained samples extracted, amplified and typed at the scene. A DNA profile search against a mock CODIS database with a "convicted offender" sample was successfully conducted within six hours of crime scene arrival. This successful demonstration of on-site STR typing at a crime scene validates the feasibility of real-time forensic identification of humans.

In the PCR-CE microdevice discussed above, a traditional cross injector was employed which produced successful results despite its poor injection efficiency. To address this issue, Yeung et al. developed an integrated device for STR sample cleanup and separation using a method that employs a streptavidin capture gel chemistry coupled to a CE separation channel for forensic STR analysis [44]. Compared with conventional microchip CE with a cross-injector, the fluorescence intensity could be improved 10–50-fold for monoplex samples, and 14–19-fold for 9-plex STR products. This capture structure can be readily incorporated into the high-throughput μCAE and integrated PCR-CE microdevices with the aim to significantly increase the sensitivity, robustness and data quality of low-copy-number and degraded DNA analysis.

Conclusions and Prospects

Over the past two decades, microfluidic devices for genetic and gene expression analysis have advanced rapidly. Most of the analytical steps have been successfully translated into chip formats where they demonstrated at least ten-times better performance over conventional counterparts. However, thus far, microfluidic systems are primarily utilized by the academic research community. We believe fully integrated microfluidic systems that contain all the necessary analytical components and provide a complete solution to users will ultimately find wide application because these integrated systems provide extraordinary advantages which are absent in discrete microdevices with single functions. The reduced reaction volume expedites the assay and enhances the sensitivity; precise fluidic control coupled with efficient sample transport prevents sample loss or dilution and increases assay sensitivity; and automated operation saves labor and time while eliminating the risks of contamination. While robotics provides macro-scale integration of analytical processes, microfluidics provides micro-scale integration and should be thought of as a microfabricated liquid robot operating at the pL–nL volume scale.

Considering potential needs in the future, several promising "killer applications" are emerging. First, portable genetic analysis instruments are available with sample in–answer-out capability for on-site identification of humans or pathogen detection. This application is timely because biothreats are of increasing concern for military and civilian populations. Second, integrated DNA sequencing systems which can reduce the total cost of *de novo* sequencing by two orders of magnitude (down to $100,000 genome) will yield great benefits to all genome-related research. Third, microfluidic systems with single-molecule or single-cell sensitivity will enable pioneering genetic

studies of somatic variation in stem cell and cancer research.

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