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Review

Bioanalysis in microfluidic devices

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Abstract

Microfabricated bioanalytical devices (also referred to as laboratory-on-a-chip or μ-TAS) offer highly efficient platforms for simultaneous analysis of a large number of biologically important molecules, possessing great potential for genome, proteome and metabolome studies. Development and implementation of microfluidic-based bioanalytical tools involves both established and evolving technologies, including microlithography, micromachining, micro-electromechanical systems technology and nanotechnology. This article provides an overview of the latest developments in the key device subject areas and the basic interdisciplinary technologies. Important aspects of DNA and protein analysis, interfacing issues and system integration are all thoroughly discussed, along with applications for this novel ''synergized'' technology in high-throughput separations of biologically important molecules. This review also gives a better understanding of how to utilize these technologies as well as to provide appropriate technical solutions to problems perceived as being more fundamental.
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Keywords: Reviews; Chip technology; Instrumentation; DNA; Proteins

Contents

1. Introduction 160
2. Genomic applications 160
   2.1. Separation media 160
   2.2. Double- and single-stranded DNA analysis and genotyping 161
   2.3. Sequencing and other applications 161
   2.4. Polymerase chain reaction 163
3. Protein analysis on-chip 164
   3.1. Separation and system integration 166
   3.2. Immunoassays and other specific applications 167
   3.3. Microchip and mass spectrometry 168
4. Analysis of other biologically important molecules 169
5. Interfacing microchips with macroworld for high-throughput applications 170
6. Integrated microdevices and other novel applications 173

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7. Fantastic plastic 176
8. Conclusions 179
Acknowledgements 181
References 181

1. Introduction

In the last decade microfabrication technologies, originally developed for the production of silicon-based chips for the microelectronics industry, have spread out in a variety of applications as chemical and biochemical analysis tools. Microfluidic devices are used for transporting and manipulating minute amount of fluids and/or biological entities through microchannel manifolds allowing integration of various chemical and biochemical processes into fast and automated monolithic microflow systems. The various types of microfabricated chemical and biochemical devices are often referred to as laboratory-on-a-chip systems, which encompass miniaturized separation systems, microreactors, microarrays, combinations of the above, etc. One of the latest hallmarks of the rapid progress in this field is several recently published books [1–3] describing in detail the broad multidisciplinary subject of microfluidics, which has roots in different areas of physics, chemistry, biology, and engineering. Materials and microfabrication technologies, currently used to make microfluidic devices, as well as the main principles and formats of typical electrokinetic manipulations, separations and detection methods, have been described in detail in several reviews [4,5] and references therein. The topics covered in this review are limited to the most recent developments and trends in microfluidics-based analysis of biological entities, mostly biopolymers, such as nucleic acid, protein and carbohydrate molecules. The progress in this area has been focused on the rapidly developing fields of genomics, proteomics and metabolomics. It has become evident that there is a tremendous market potential for microdevices aiding in diagnostics, drug discovery and evaluation of new pharmaceuticals, since these devices are expected to satisfy the urgent demand for high-throughput and large-scale applications. The main advantages of miniaturizing chemical and bioanalytical tools include improved performance, speed and throughput, reduced costs and reagents consumption, and the possibility of parallel and integrated analysis, as discussed in numerous reviews [4,6–9].

2. Genomic applications

Separation of nucleic acids is one of the leading applications of microchip-based analysis today. Due to the negligible deteriorating effects of Joule heating during electric field mediated separations in microchannels and the ability to inject very small, well-defined sample plugs, the resolving power of microfluidic bioanalytical devices are mainly diffusion-limited resulting in superior performance compared to slab gel and capillary electrophoresis. Rapid, high-quality separations on-chip have been demonstrated for the analysis of oligonucleotides, RNA and DNA fragments, as well as for genotyping and sequencing applications. Typically, laser-induced fluorescence (LIF) is employed for detection, but other methods such as mass spectrometry (MS) and electrochemical detection have been investigated as well. The latter is readily adaptable for miniaturization and on-chip integration [10–12]. Among other examples are Raman spectroscopy [13] and holographic refraction index detection [14].

2.1. Separation media

The shorter separation distances, compared to that used in conventional capillary electrophoresis (CE), represent new challenges to the optimization of separation conditions, such as electrokinetic manipulations, channel geometry and sieving media. The geometrical effects of folded microchannel structures on band broadening have been extensively studied [15,16]. A variety of DNA separation matrices developed for CE instruments is based on viscous solutions of entangled water-soluble polymers, successfully applied to microchip electrophoresis. Linear polyacryamide and its derivatives, polydi-

methylacryamide, polyethylene oxide, polyvinylpirrolidone, polyethylene glycol with fluorocarbon tails, hydroxyethylcellulose and various cellulose derivatives, other polysaccharides, etc., have been used for size separation of nucleic acid molecules in capillaries [17], and some of these matrices have also been adapted for microchip electrophoresis. Recently introduced novel thermoresponsive co-polymers, comprising hydrophobic and hydrophilic blocks, such as pluronics [18–20] and polyisopropylacrylamide grafted with polyethylene oxide chains [21], have also shown promising results. These matrices have a pronounced temperature-dependent viscosity transition point which suggests promising implementations. In particular, thermoresponsive polymers can offer some practical advantages for microchannel electrophoresis, enabling easier handling and loading of the viscous polymer solutions without the requirement of a high-pressure manifold. Buchholz et al. have constructed interesting ''viscosity switch'' materials which response to changes of temperature, pH or ionic strength [22]. These matrices are based on co-polymers of acrylamide derivatives with variable hydrophobicity and possess a reversible temperature-controlled viscosity switch from high-viscosity solutions at room temperature to low-viscosity colloid dispersions at elevated temperatures. Also, high resolving power and good DNA sequencing performance (463 bases in 78 min) was achieved with these sieving media. Another family of thermoassociating matrices has been introduced by Sudor et al. [23]. These comb polymers are made of hydrophilic backbone (polyacrylamide) grafted with lower critical solution temperature side chains (poly-*N*-isopropylacrylamide). Thermothickening properties of these polymers, due to formation of transient intermolecular cross-links at higher temperatures, offer clear advantages for DNA sequencing, e.g., easy handling of low-viscosity solutions at low temperatures and excellent sieving characteristics of high-viscosity solutions at elevated temperatures.

An example of matrix-free approach to DNA separations has been presented by Han and Craighead [24]. A nanofluidic channel was designed and fabricated to separate long DNA molecules based on the so-called ''entropic traps'' principle. The separation channel with wide (1.5–3 μm) and narrow (75–100 nm) regions caused size-dependent trapping of DNA at the onset of each constriction (Fig. 1). The mobility differences created by this means, enabled DNA separation without the use of a sieving polymer matrix or pulsed electric fields. Fragments in the 5–160 kilobase pairs (kbp) range were successfully separated in a 1.5 cm channel. A device with parallel channels was also demonstrated. Simplicity of the microfabrication approach, its efficiency and the possibility of theoretical modeling suggested potential practical applications of these results into an integrated DNA analysis system.

2.2. Double- and single-stranded DNA analysis and genotyping

Major applications of electrophoresis microchips include sizing of double-stranded DNA fragments [5,25–29], short single-stranded oligonucleotides [30] and ribosomal RNA fragments [31]. High separation performance and speed (a few minutes range) have been achieved. DNA genotyping on microchips enables quick identification of genes and can substantially enhance the capabilities of genomic, diagnostic, pharmacogenetic, and forensic tests. The identification of genes related to heredity diseases, such as muscular dystrophy [32] and hemachromatosis [33], has been successfully performed on-chip. An ultra-fast allelic profiling assay for the analysis of short tandem repeats (STRs) has also been demonstrated [34]. Separation of CTTv quadruplex system was accomplished in less than 2 min using 26 mm separation distance, which is 10- and 100-times faster than capillary or slab gel electrophoresis, respectively [35]. Two-color multiplex analysis of eight STRs loci has also been performed by the same group.

2.3. Sequencing and other applications

A number of publications has proved the feasibility of DNA sequencing in microfluidic chips. This is one of the most challenging tasks in DNA electrophoresis, since very high resolving power is required for long reads and accurate base calling. High-speed, high-throughput four-color sequencing analysis has been demonstrated on-chip in 20 min with read-length over 500 bases [36]. Microfabricated devices

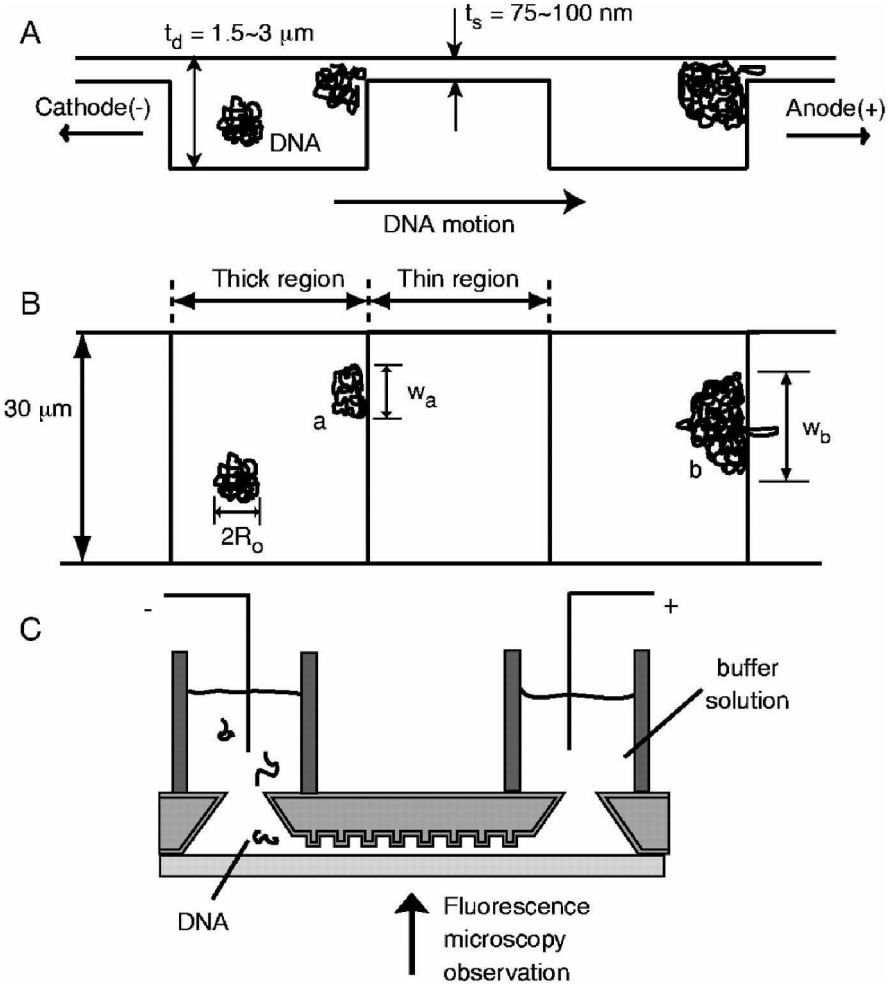

Fig. 1. Nanofluidic separation device with entropic traps. (A) Cross-sectional diagram. Electrophoresed DNA molecules are trapped whenever they meet a thin region with the depth (t_s) much smaller than their radius of gyration (R_o). t_d – Depth of the larger channel regions. (B) Top view of the device during operation. Trapped DNA molecules eventually escape, with a probability that is proportional to the length of the slit covered by the DNA molecule (w_a and w_b). Larger molecules have higher escape probability because they cover wider regions of the slit ($w_b > w_a$). (C) Experimental setup. From Ref. [24] with permission.

can perform high-throughput DNA sequencing using 96-channel array format [37]. Based on the best results reported so far, roughly 1200 bases per channel per hour is possible, which would give the yield of 2.7 Mbases per day in a 24 h cycle, using 96-lane chips [38].

A combination of microfluidic capabilities and sensitive fluorescence-based detection methods enables detection of a single DNA molecule on CE microchips. A single molecule fluorescence burst counting technique has been used to monitor DNA separations in microfluidic channels, employing avalanche photodiode detector and sample stream focusing either electrodynamically or by physical tapering of the separation column in the detection region [39].

DNA field-inversion electrophoresis has been implemented on a microchip device to accommodate a shorter effective separation length [40]. The method employed a periodically switched pulsed electric field at a 180° angle with a certain optimized frequency, at which DNA molecules exhibit a

mobility minimum and band inversion phenomena. Short oligonucleotides, 20-, 40- and 60-mers, were effectively separated, using only 6 mm separation distance. The authors suggested that this technique might have a great potential for single-strand conformation polymorphism (SSCP) analysis.

An interesting approach to reduce the applied separation voltage, maintaining yet high electric field strength, has been proposed [41]. An array of microfabricated electrodes divided a channel into smaller separation zones. A small d.c. voltage was sequentially applied to the proper pairs of electrodes to generate a moving electric field. Different electrode layouts and their effects on the field uniformity was investigated. This technique contributes to the versatility of tools for microfluidic manipulations.

2.4. Polymerase chain reaction

Since DNA amplification by polymerase chain reaction (PCR) has become a key tool in modern molecular biology, a number of groups have investigated microchip-based PCR systems in both disposable microchamber and microfluidic formats. The obvious benefits of such miniaturization come from the improved thermal energy transfer compared to conventional macrovolumes, resulting in a greatly increased speed of thermal cycling, reduced amount of expensive reagents used, and the possibility to create versatile, multifunctional integrated systems. However, potential problems, associated with PCR downscaling, such as surface related effects, become greatly enhanced with shrinking reaction volumes and consequently increasing surface-to-volume ratio. Various biocompatible PCR-friendly surfaces have been investigated by passivation of silicon, fused-silica, glass or plastic walls via coatings by chemical modification and/or physical adsorption. The other issue is related to handling of small volumes of liquids and the incorporation of miniaturized DNA amplification unit into the chain of sample processing and analysis, to develop truly automated and high throughput technology. The major milestones of the development in this field have been discussed elsewhere [6,8]. This review concentrates only on a few recent interesting implementations. A number of studies have been done to optimize microscale DNA amplification. Two possible embodiments can be envisioned, such as closed microchamber and open format, both of which can be multiplexed to an array. Fast real-time PCR analysis was demonstrated in silicon microchambers by several groups [42–45]. DNA amplification can be coupled with special microfluidic cartridges designed to carry out several sequential steps of DNA extraction from different sample types (tissues, cells, whole blood, soil, food, etc.) prior to the PCR [46]. DNA amplification in open format has not yet been reported, but other chemical and enzymatic reactions, conducted in nano- and picovials, have shown promising results [47–50].

There have been several reports on integration of DNA amplification and electrophoretic separation on a single microfabricated chip [51–56]. These devices contained small reaction wells, which were thermocycled to generate the amplicons, followed by the injection/separation/detection steps in the interconnected microchannel network. An integrated system combining fast on-chip DNA amplification by local thermocycling and preconcentration technique, followed by microchip electrophoretic sizing, was reported in Ref. [54]. DNA fragment preconcentration was accomplished prior to injection by means of a microfabricated porous membrane structure (Fig. 2). This method enabled reduction of the PCR cycle number and speed up the total analysis time to less than 20 min. A microchip device, developed by Woolley et al., was coupled to a special microchamber with fast heating/cooling capabilities for effective DNA amplification [51]. Recently the same group developed an integrated monolithic system incorporating several 280 nl PCR chambers etched into a glass structure, connected to microfluidic valves and hydrophobic vents for sample introduction and immobilization during thermal cycling (Fig. 3) [55,56]. The low thermal mass and use of thin-film heaters enabled as fast cycle times as 30 s. The amplified products were directly injected into the corresponding gel filled microchannels and detected by laser-induced fluorescence. The excellent detection sensitivity attained (20 copies/μl) suggested that it might be possible to perform stochastic single molecule PCR amplification.

It is interesting to note similar trends of system integration in capillary electrophoresis. An automated capillary array machine for DNA typing

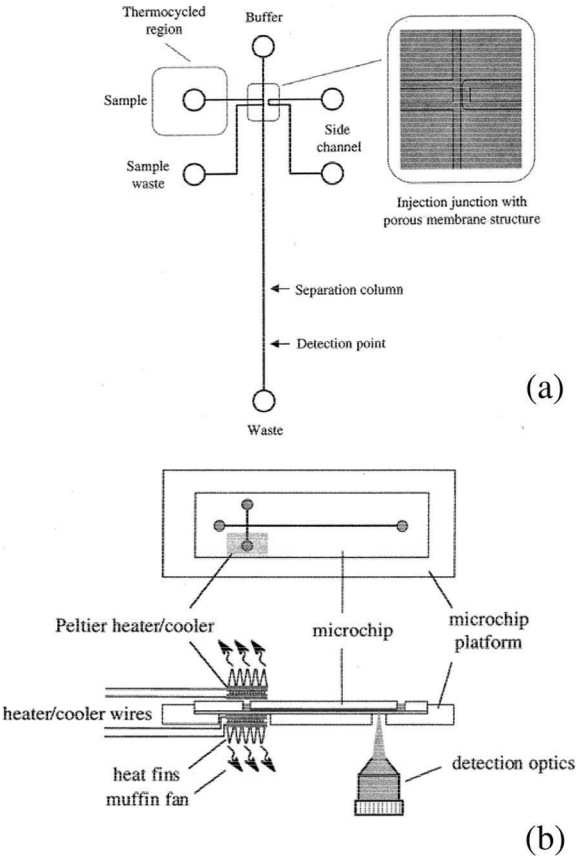

Fig. 2. Integrated system for rapid PCR analysis on-chip. (a) Microchannel design with a porous membrane structure incorporated in the injection valve for DNA fragment pre-concentration prior to injection and separation. (b) Schematics of the dual thermoelectric assembly for fast thermal cycling followed by microchip electrophoresis. From Ref. [54] with permission.

directly from blood samples [57], as well as nano-reactor-based cycle sequencing instrument [58], have been developed by Yeung and co-workers. Microfluidic elements and a microthermocycler were incorporated to co-amplify STR loci or carry out sequencing reaction, as well as on-line capillary zone electrophoresis (CZE) purification of the products, loading/injection, and regeneration/cleaning system.

An interesting alternative to a microchamber reaction format has been proposed by Kopp and co-workers, who demonstrated a continuous-flow PCR system [59,60]. A PCR cocktail was pumped continuously through a serpentine glass channel, periodically passing the three different temperature zones to perform denaturation, annealing and amplification steps. Although, total reaction time was relatively long (50 min for 20 cycles), multiple simultaneous reactions can be carried out by sequential introduction of separate reactions in each loop.

Ultra-short thermal cycling times of 17 s per cycle have been achieved by Landers and co-workers using IR radiation-mediated heating and compressed air cooling to amplify DNA in a capillary or on-chip microchambers [61–63]. A tungsten lamp, thermocouple feed-back and computer interface were used to accurately control the temperature by the combination of light intensity and air flow.

A novel thermocycling system, based on a capillary equipped with bi-directional pressure-driven flow and in situ optical position sensors, has been presented by Chiou et al. [64]. A 1 μl PCR mix droplet was controllably moved between three different heated zones in a 1 mm I.D. oil-filled capillary. A light scattering detector was used to control the droplet position. Thirty PCR cycles were completed in 23 min with a good amplification efficiency. The maximum possible speed with this arrangement was theoretically estimated as high as 2.5 min.

A hybrid poly(dimethylsiloxane) (PDMS)–glass microchip for functional integration of DNA amplification and gel electrophoresis was recently reported in [65]. Thermoelectric heating/cooling control was used in this case. Such devices can be disposable due to their inexpensive and relatively simple fabrication.

3. Protein analysis on-chip

The conventional approach to analyze protein molecules typically comprises their extraction from cells, separation and detection by one- or two-dimensional (2D) gel electrophoresis, bands cut-out and in-gel digestion, followed by MS analysis of the resulting peptide mixture. These traditional techniques are slow and labor intensive. Fast and high-throughput integrated multisample systems are in great demand. Microfluidic devices offer new opportunities which were unavailable in traditional protein analysis technologies, with the potential to control and automate multiple sample processing steps. The majority of recent developments in microfluidics for protein applications has been related to the combina-

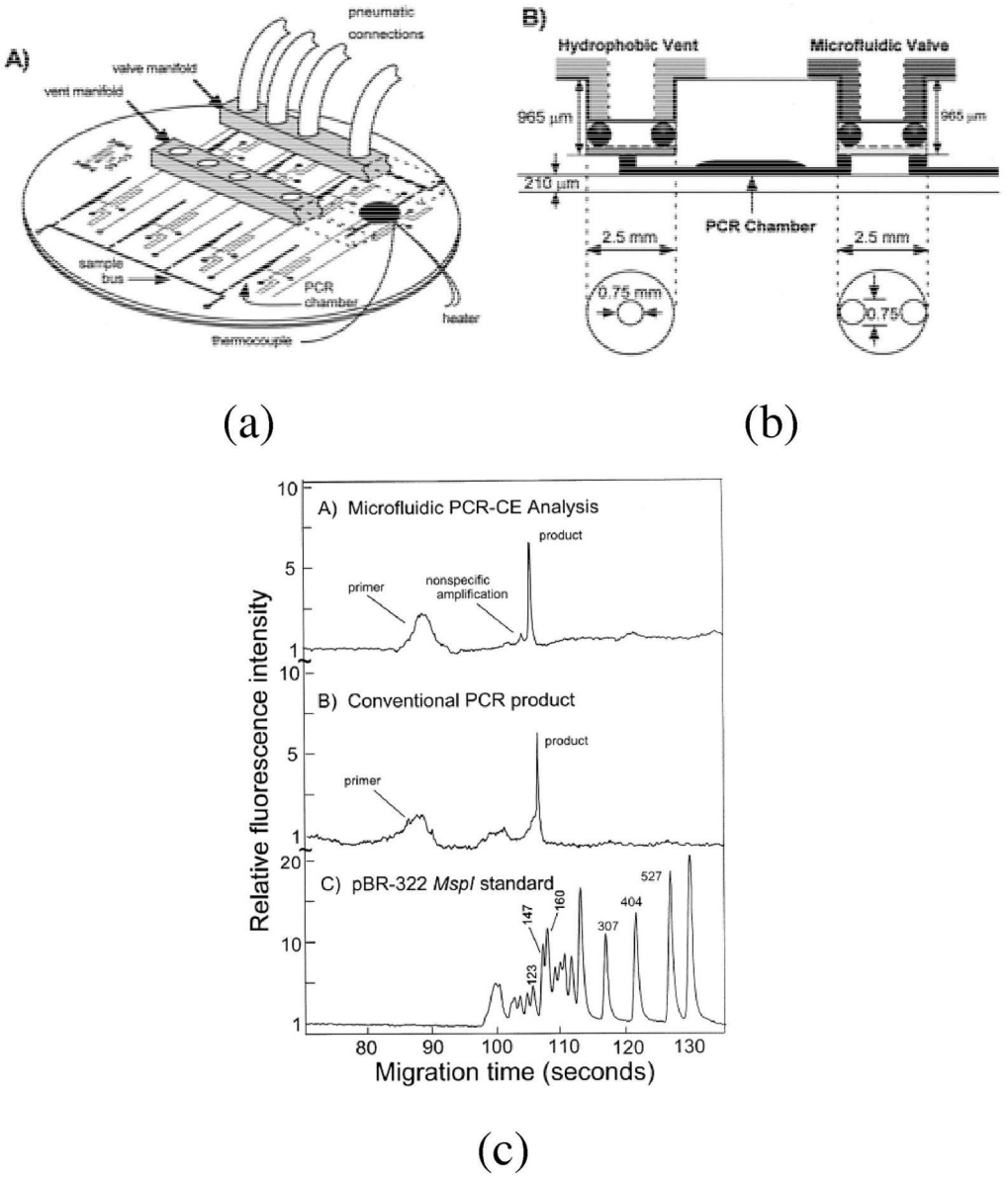

Fig. 3. Monolithic integrated microfluidic PCR amplification and CE analysis system. (a) Schematics of PCR–CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents are used to locate the sample and to eliminate gas. The chambers are directly connected to the cross of the microchannel system for product injection and analysis. Two aluminum manifolds, for the vents and valves, are placed into the respective ports and clamped in place using vacuum. The manifolds are connected to an external solenoid valve for pressure and vacuum actuation. Thermocycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nl reaction chamber. (b) Detailed schematics of microfluidic valves and hydrophobic vents. Sample is loaded from the right by opening the valve by vacuum and forcing the sample under the membrane by pressure; vacuum is simultaneously applied at the vent to evacuate the air from the chamber. The sample stops at the vent, and the valve is pressure-sealed to enclose the sample. Dead volume for the valves and vents is approximately 50 nl. (c) Microfluidic PCR–CE analysis of an M13 amplicon (20 starting copies/μl). 20 cycles of 95°C for 5 s, 53°C for 15 s, and 72°C for 10 s. Electropherograms: upper trace: chip PCR–CE analysis; middle trace: positive control with identical PCR mix using a Peltier thermal cycler; lower trace: pBR322 *Msp*I DNA ladder for size comparison. From Refs. [55,56] with permission.

tion of single or multidimensional microchannel separations and MS detection [66]. Here we discuss only a few examples of the most recent microfluidics-based solutions in the rapidly developing proteomics world.

3.1. Separation and system integration

A number of research groups has been focusing on the realization of traditional 2D gel electrophoresis on microchip format. The problem of protein purification and desalting prior to the analysis was addressed in microfabricated structures using semipermeable membranes, sandwiched between microfluidic manifold layers and connecting the buffer and analyte counterflows [67,68]. Such microdialysis enabled effective cleanup of analytes of interest from low molecular mass compounds, and enhanced the following MS characterization. Similar fluidic constructions, microfabricated in plastic [68,69], are discussed in more detail later in this paper (Section 7).

Examples of isoelectric focusing (IEF) of proteins in microchips are described in Ref. [66] demonstrating the potential of microscale techniques. Chip-based IEF has been accomplished in 30 s in 7 cm channel with a peak capacity of about 30–40 peaks, as reported in Ref. [70]. Electroosmotically driven mobilization of the focused zones was found the most suitable technique for the microchip approach due to its easy implementation and high speed. Nevertheless, IEF analysis has been performed so far on simple samples only, and the resolving power achieved was low in comparison with conventional immobilized pH gradient IEF gels. An integrated IEF–electrospray ionization (ESI) MS plastic microfluidic device, coupling electrospray tip to the IEF microchip, has also been reported [71].

Chromatography and electrophoresis represent the two major separation techniques for proteins. The latter is by far more popular in microchip applications due to its easier realization in miniaturized formats. Nevertheless, on-chip chromatographic separations have recently been attempted by trapping coated beads into a microcavity within a microchannel network [72], or using in situ microfabricated [73] or polymerized [74] beds.

Numerous examples of microchip electrophoretic separations of peptides and proteins in free zone and sodium dodecyl sulfate (SDS) electrophoresis format have been demonstrated and reviewed [4,6,8,66]. SDS gel electrophoresis has also been performed in microchannels by Yao et al. [75], separating three proteins in M_r 9000–18 000 range in 35 s (Fig. 4), which is fivefold quicker than the fastest separation reported so far in CE [76].

The development of more sophisticated microfluidics systems with both ''horizontal'' integration by building parallel analysis lanes for high throughput applications, and ''vertical'' integration by implementing several functions on a single device, is perhaps the most exciting trend in the microchip world. Recently, a new microfluidic glass device was developed for integrated protein sizing assay, which performs separation, staining, virtual destaining and detection steps, and can sequentially analyze 10 different samples in less than 30 min [77]. Universal non-covalent fluorescent on-chip labeling was employed in combination with post-separation dilution to reduce fluorescent background associated with SDS micelle-bound dye to increase the signal-to-noise ratio. Non-covalent fluorogenic dyes, such as Sypro dyes, Nile Red, ethidium bromide, etc., bind to hydrophobic regions of the SDS–protein complexes, resulting in fluorescence enhancement, similar to that of intercalating dyes in DNA analysis, and offer certain attractive features for protein labeling over traditional chemical attachment [78]. The applicability of this technique to microscale protein separations has been investigated by several research groups [79–81]. High speed and sensitivity of microseparations, and the option of on-chip pre- or post-column labeling, mixing, destaining, etc., proved to be clearly beneficial in combination with non-covalent protein staining dyes. High separation efficiency and good detection limits (as low as pg and amol level in some cases [80]) were demonstrated.

An ultimate microfabricated analogue to 2D IEF–SDS–polyacrylamide gel electrophoresis (PAGE) platform has not yet been reported, however, examples of two dimensional approaches in microchips have been recently published. Multidimensional separations are attractive due to the multiplicative increase in peak capacity over one-dimensional methods. Open channel electrochromatography (OCEC) and capillary electrophoresis, as first and

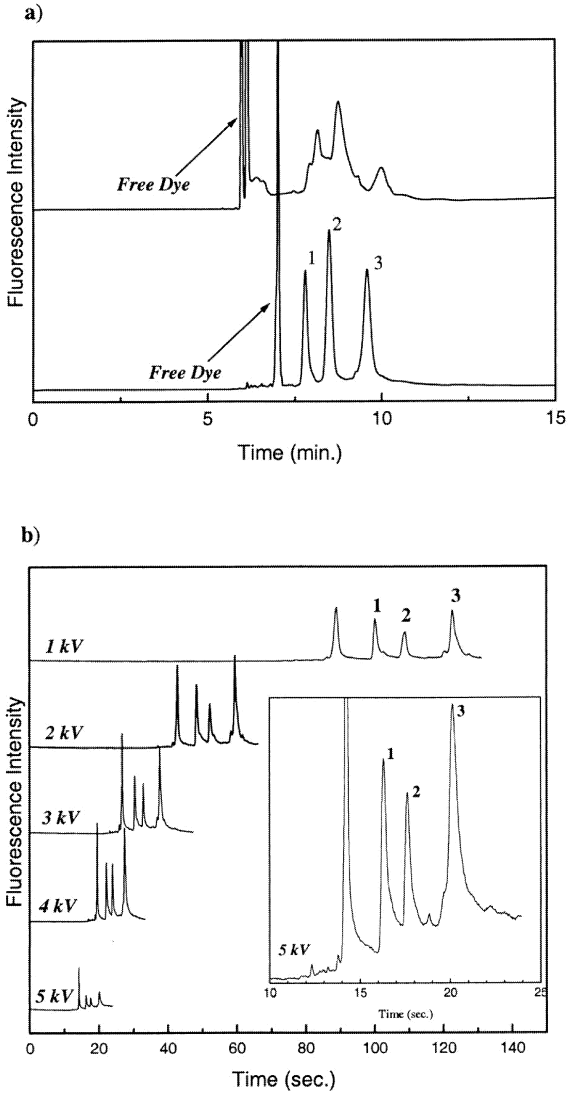

Fig. 4. Electropherograms of three cysteine containing fluorescently labeled proteins: calmodulin, M_r 9000 (1); lysozyme, M_r 14 500 (2); and staphylococcal nuclease, 16-cys mutant, M_r 18 000 (3). (a) Capillary-based SDS–capillary gel electrophoresis with the proteins labeled non-specifically with fluorescein succinimidyl ester (upper trace) or labeled specifically with fluorescein-maleimide (lower trace). Capillary: 20 cm (27 cm total length)×100 μm I.D. uncoated fused-silica. Separation voltage 8.1 kV, injection 4 kV for 30 s. (b) Microchip-based SDS–capillary gel electrophoresis of the specifically labeled proteins with different separation voltages. The concentration of each protein in the mixture was estimated to be $1\cdot10^{-9}$ to $10\cdot10^{-9}$ *M*. From Ref. [75] with permission.

second dimensions, respectively, have been implemented on a single glass chip [82]. A 25-cm spiral OCEC channel, coated with octadecyl groups, was connected with a 1.2-cm straight CE channel through a cross injection junction. The effluent from the first dimension was repeatedly injected into the CE channel every few seconds. The sampling (injection) rate of the effluent of the first dimension into the second one was optimized to perform representative CE analysis of the OCEC separation products. Fluorescently labeled tryptic digest of β-casein was analyzed in 13 min in this device. Another, similar 2D microfabricated device [83] combined micellar electrokinetic chromatography (MEKC) and CE for the analysis of peptide mixtures (Fig. 5). The peak capacity of the 2D device was estimated to be in the 500–1000 range, greatly improving the separation efficiency of each dimension alone. The approach can be useful for rapid automated fingerprinting of proteins and peptides with possible coupling to MS detection.

3.2. Immunoassays and other specific applications

Immunoassays represent a very important tool in clinical diagnostics and biomedical research and industry. Various miniaturized immunoassays mostly based on competitive antigen–antibody interactions, were successfully performed in electrophoresis microchips, demonstrating greater speed and feasibility of automated analysis in a portable format. Earlier progress in this field has been described in several reviews [8,66]. Among the most recent developments, Gottschlich et al. [84] reported an integrated microchip device that sequentially performed enzymatic reactions, electrophoretic separation of the products, post-separation on-line labeling of the peptides and proteins, followed by fluorescent detection. A tryptic digestion of oxidized insulin B-chain was performed in 15 min under ‘‘stopped flow’’ conditions in a locally thermostated channel. The electrophoretic analysis was completed in 1 min, and the separated peptides were labeled with naphthalene-2,3-dicarboxaldehyde (NDA) by on-chip mixing. The same device was also used for on-chip reduction of disulfide bridges in insulin.

A new class of microfluidic immunoassays is based on solid supported lipid bilayers [85]. The

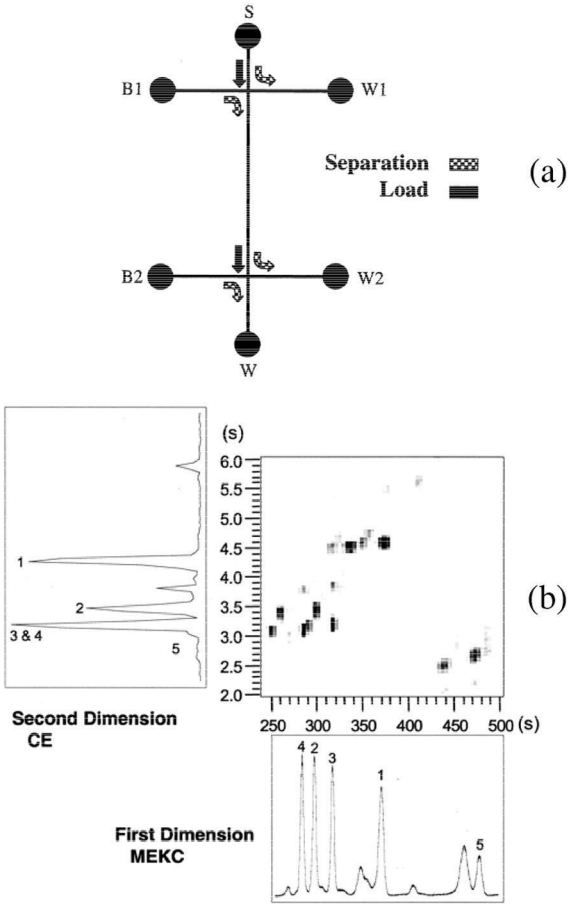

Fig. 5. (a) Schematic diagram of a two-dimensional microchip, illustrating the direction of fluid flow during sample loading (solid arrows) and separation modes (hatched arrows). (b) One- and two-dimensional separations of fluorescein isothiocyanate-labeled peptide standards. Effluent from the first dimension is injected in every 4 s into the second dimension. Peaks: 1=leucine enkephalin, 2=angiotensin I, 3=angiotensin III, 4=neurotensin, 5=bradykinin. From Ref. [83] with permission.

bilayers, created on PDMS surfaces of an array of parallel microchannels, contained dinitrophenyl (DNP)-conjugated lipids for binding with fluorescently labeled anti-DNP antibodies of different concentration in each channel. The methodology can be used for performing rapid and accurate heterogeneous assays in a single experiment, and the amount of proteins required is significantly reduced compared to conventional methods.

Nanovials are another example of the use of microfabricated devices for protein analysis [47,48]. These very small wells, fabricated in different substrates, feature high surface-to-volume ratio and have demonstrated increased reaction efficiency (e.g., enzymatic digestion), compared to conventional microfuge vials. The solvent evaporation problem was addressed by periodic solvent addition and/or humidification of the surrounding environment, as well as continuous compensation for evaporation using an array of solvent-delivery capillaries [47]. Performing sub-nanoliter scale chemistry in a parallel fashion opens new opportunities in high throughput proteomics, particularly when combined with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS.

3.3. Microchip and mass spectrometry

Since MS is one of the most frequently used methods of choice in protein analysis and characterization, a number of successful efforts have been reported to couple microfluidic devices to mass spectrometers [66,71,86–92]. Such combinations enabled automated sample delivery and enhanced MS analysis efficiency by, for instance, sample processing/enrichment/cleanup and fractionation prior to the detection. These devices can transport the analyte fluid electrokinetically or by pressure, and generate electrospray via an attached capillary or more complex emitter couplings. Enzymatic digestion was monitored by peptide mass fingerprinting in real time with high detection sensitivity (0.1–2 pmol/μl of loaded sample) on a hybrid microchip nanolectrospray device [91]. The same research group used a microfabricated, electrically permeable thin glass septum to generate electrospray by electroosmotically pumping of the solutions through field-free channels, past the point where CE electric potential was applied [92]. Automated MS analysis was demonstrated by Ekstrom et al. [93], utilizing a porous microfabricated digestion chip, integrated with sample pretreatment robot and microdispenser for transferring the reaction products to a MALDI target plate.

A new design for high-throughput microfabricated CE–ESI-MS with automated sampling from a microwell plate has been reported by Zhang and coworkers [94,95]. The assembly combined a sample

loading port, separation channel, and a liquid junction for coupling the device to the mass spectrometry system with a miniaturized subatmospheric electrospray interface (Fig. 6). The microdevice was attached to a polycarbonate manifold with external reservoirs equipped for electrokinetic and fluid pressure control, which simplified the microdevice design and enabled extended automatic operation of the system. A computer-activated electro-pneumatic distributor was used for sample loading and channel washing. Automated CE–ESI-MS analysis of peptides and protein digests was successfully demonstrated.

4. Analysis of other biologically important molecules

Compared to nucleic acids and proteins, applicability of microfluidic devices to the analysis of

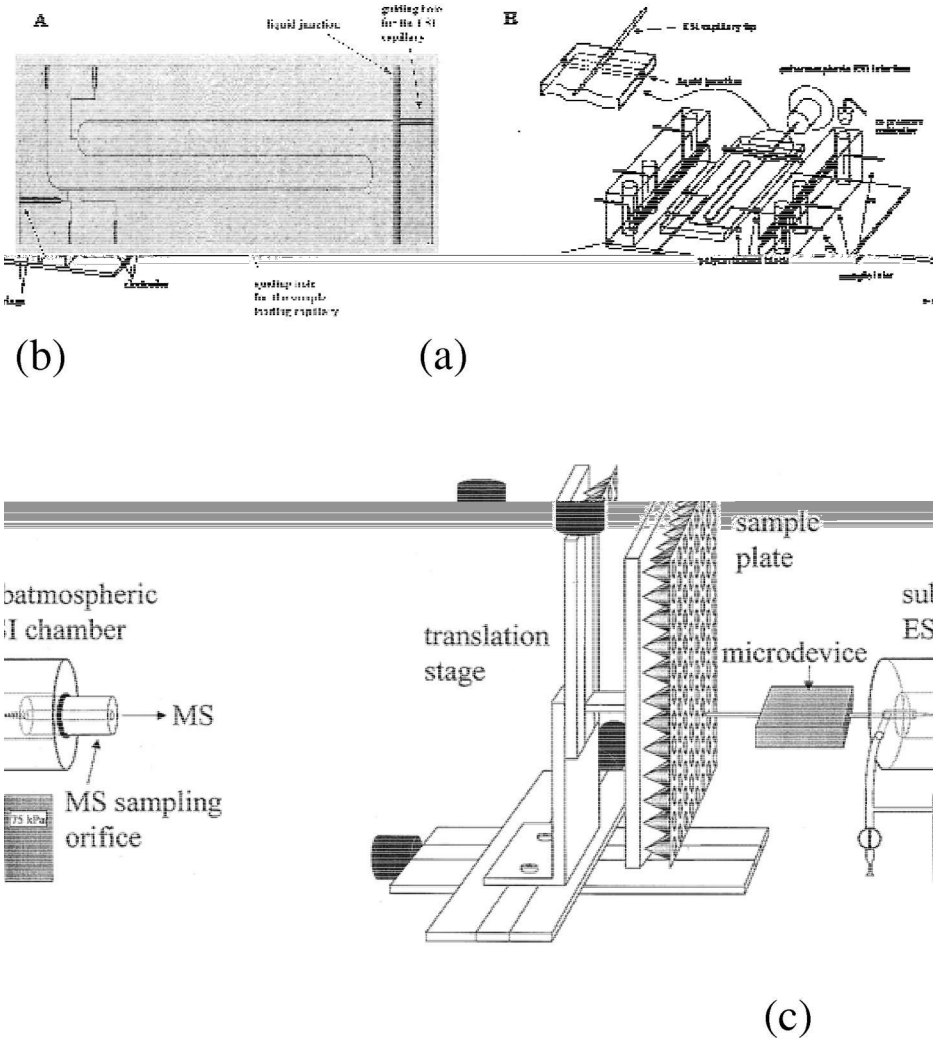

Fig. 6. Automated high throughput microchip electrophoresis ESI-MS system. (a) Photograph of the glass microchip (5×2 cm). (b) Diagram of the microdevice design, including the microchip and polycarbonate manifold. (c) Overall design of the coupling of the microwell plate sample delivery system equipped with a microdevice for high-throughput separation–MS analysis. From Ref. [95] with permission.

other biologically important macromolecules, such as carbohydrates, lipids and fatty acids has been very sparse so far, probably due to the limited development of CE techniques for these analytes [96]. Fractionation of lipids and fatty acids is complicated mainly because of their poor solubility in aqueous buffers, apparently, requiring micellar systems or non-aqueous organic solvents. On the other hand, carbohydrate analysis by CE is very complex due to wide diversity of oligosaccharide structures [97] and the necessity of derivatization to introduce charges and UV or fluorescent labels into the neutral sugar molecules.

A mixture of sucrose, *N*-acetylglucosamine, and raffinose was separated in 17 s on synchronized cyclic capillary electrophoresis chip with a square channel, using a holographic-based refractive index detection method [14]. Although the initial detection limits were poor compared with alternative techniques, a potential of a small volume universal detection for chip-based separations was demonstrated.

Wang et al. [98] demonstrated simultaneous microchip CE-based bioassays of a mixture of metabolites (glucose, uric acid, ascorbic acid and acetaminophen). Enzymatic oxidation of glucose was carried out in a microchannel, followed by the separation and electrochemical detection of the neutral peroxides and anionic uric and ascorbic acids. The chip-based protocol allowed integration of miniaturized bioassays, separations and amperometric detection on a single device, competitive with bench-top analyzers and conventional biosensors in terms of performance, speed, sample volume, and size. In spite of lack of more examples of microchip applications for the analysis of these biopolymers, it is reasonable to anticipate its growth in the near future.

5. Interfacing microchips with macroworld for high-throughput applications

In research-based industries, such as genomics, proteomics, metabolomics and especially drug discovery, the need to carry out a large number of complex experiments poses a real challenge in terms of efficiency, data quality and cost of analysis. For example, in primary drug screening alone, major pharmaceutical companies test-score new biological targets against compound libraries of hundreds of thousands of compounds per year, generating millions of data points in search for new targets. Therefore, high throughput systems that perform large-scale experimentation and analysis, using minor amounts of reagents, are in great demand. The real bottleneck in microscale analysis is to find the proper interface between the chip and the external world. This is often required to automate sample introduction/collection process for high throughput applications and to provide continuous monitoring of the analyte molecules. The so-called sipper chip technology [99] addresses this issue by performing experiments in a serial, continuous flow fashion at rates of 5000 to 10 000 experiments per channel per day. The system uses capillaries to draw nanoliters of reagents from microwell plates into the channels of the chip, where they are mixed with the target biomolecules and a series of processing steps are carried out to determine if there is any effect of the compound on the target. A range of assays, including fluorogenic, mobility shift, fluorescence and cellular tests, have been demonstrated using various chip designs.

Another possible solution to this problem has been implemented recently by Attiya et al. [100]. The device contained a large sample introduction channel with a volume flow resistance $>10^5$-fold lower than that in the analysis microchannels (Fig. 7). This approach enabled interfacing the large sample introduction channel with an external pump (up to 1 ml/min flow-rate) for pressure-driven sample delivery, without perturbing the solutions and electrokinetic manipulations within the rest of the microchannel network. On-chip mixing, reaction and separation of ovalbumin and anti-ovalbumin was demonstrated with high-performance and reproducibility. Such strategy for decoupling the electrokinetic flow in the microchannels from the external environment extends the applicability of microchip analysis and provide a useful alternative to mechanical valve flow control.

Shi et al. [37] introduced a pressurized capillary array system to simultaneously load 96 samples into 96 sample wells of a radial microchannel array electrophoresis microplate for high-throughput DNA sizing (Fig. 8). As a result, 96 samples were ana-

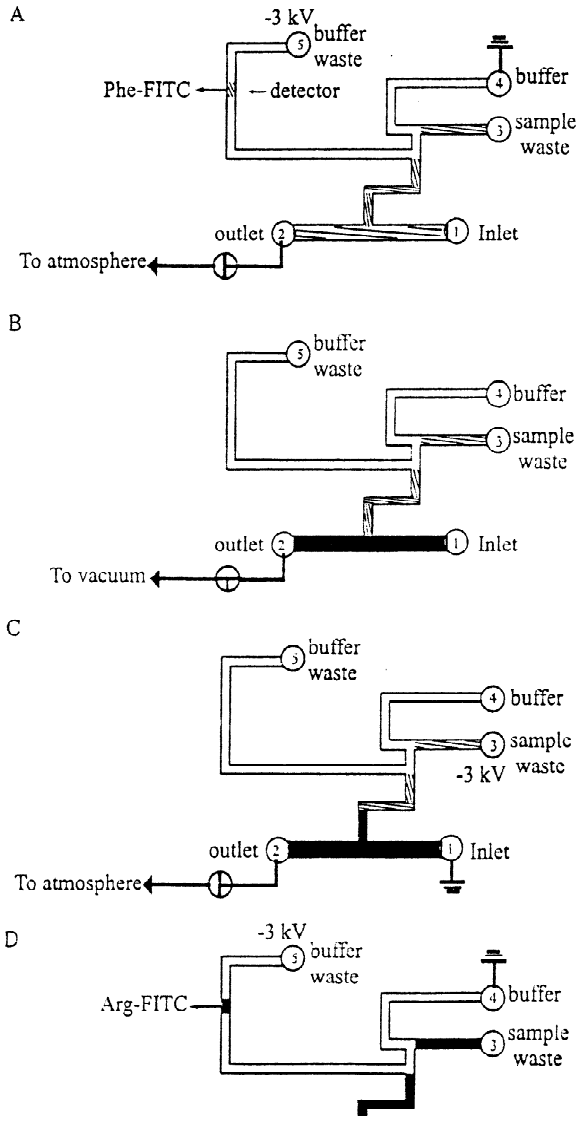

Fig. 7. Sample exchange sequence of fluorescein isothiocyanate (FITC)-labeled amino acids. (A) Switching a three-way valve to atmospheric pressure and applying −3 kV between the buffer and buffer waste reservoirs to dispense a sample plug into the separation channel. (B) The three-way valve is switched back to vacuum to remove the Phe-FITC solution from the sample introduction channel (SIC), and 6 μl of the next sample, Arg-FITC, is used to flush the SIC. (C) The valve is switched again to atmospheric pressure and another 6-μl aliquot is introduced into the SIC for analysis. Sample exchange and flushing of the SIC required 3 s, followed by application of −3 kV for 20 s to rinse the injection channel with Arg-FITC. (D) Separation mode: −3 kV between buffer and buffer waste reservoirs. From Ref. [100] with permission.

lyzed in less than 90 s per microplate, demonstrating the power of microfabricated devices for large-scale and high-performance nucleic acid characterization.

An interesting approach to perform parallel microscale separations has been developed by Hietpas et al. [101], using ultrathin slab gel electrophoresis with its parallel processing capabilities in combination with a capillary sample delivery system. The technique allowed accurate and rapid automated injection (deposition) of multiple samples (PCR products, STR fragments, oligonucleotides, amino acids) at the edge of the ultrathin slab gel for consequent separation and detection. This simple system eliminates cross intersections and additional buffer reservoirs, required for analyte injection in microchannel devices, and yields high throughput analysis with minimal need for operator intervention. Recently, the same group demonstrated continuous parallel separations in an array of separation lanes (500–700 μm wide and 250–350 μm deep) fabricated in glass chips, using the same capillary sample introduction method [102].

Membrane-mediated sample loading was introduced by Guttman and Ronai in conjunction with microscale electrophoresis using ultra-thin (≤190 μm) slab gels [103]. Minute amounts of DNA samples (0.2 μl) were spotted manually or robotically onto the surface of the tabs of a membrane loader, which was then placed into the cathode side of the cassette, in intimate contact with the straight edge of the ultrathin gel. Under the applied electric field strength, the sample DNA molecules migrated into the gel, forming finely defined sharp bands (Fig. 9). This procedure facilitated the introduction of large number of samples (96 parallel analysis lanes) into microgels, eliminating the need of forming individual wells and enabling sample loading to be accomplished easily on the bench-top, outside the separation platform. Membrane-mediated injection method is readily automated (robotic spotting) and can be applied to most high-throughput applications, such as PCR product analysis, genotyping and automated DNA sequencing [103–105].

Researchers in the drug discovery field have also been searching for microfluidic-based solutions to address the screening processes of the drastically growing scale of combinatorial chemistry libraries. The goal is a chemical microprocessor, that combines the reactions and screening on a single mi-

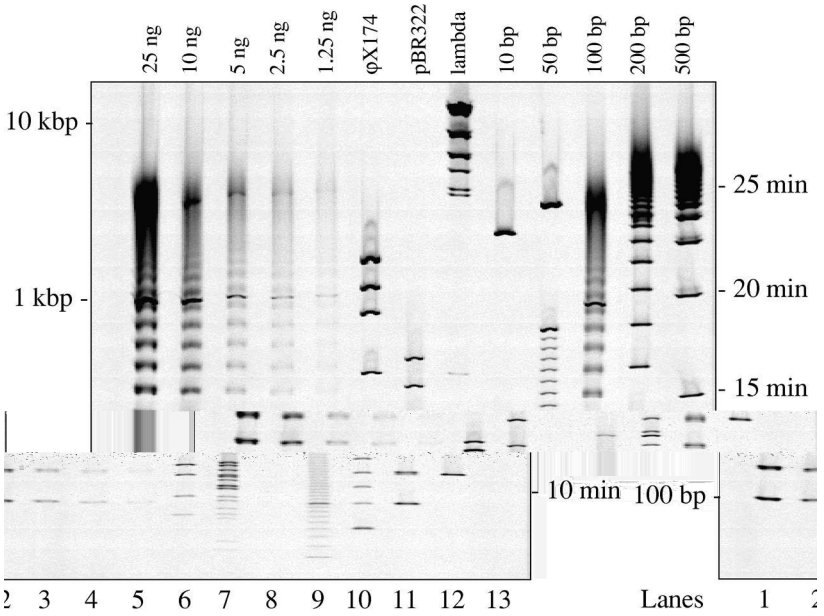

Fig. 9. Separation of DNA sizing ladders and restriction digest fragment mixtures by automated ultrathin layer agarose gel electrophoresis. Lanes: 1–5: dilution series of a 100 bp ladder (25; 10; 5; 2.5 and 1.25 ng total DNA injected); 6: ϕX174 DNA Hae-III restriction digest mixture; 7: pBR322 DNA Msp-I restriction digest mixture; 8: lambda DNA Hind-III restriction digest mixture; 9–13: 10, 50, 100, 200 and 500 bp DNA sizing ladders. Conditions: effective separation length: 6 cm; separation matrix: 2% agarose gel in 0.5× TBE buffer (45 mM Tris, 45 mM Boric acid, 1 mM EDTA) containing 25 n*M* ethidium bromide; running buffer: 0.5× TBE; applied voltage: 750 V; temperature: 25°C; Injection: membrane mediated, 0.5 μl sample per tab. From Ref. [105] with permission.

crodevice. One of the latest prominent industrial developments of the kind is a device [106], which comprises multiple reaction units on one chip. These units are enclosed but connected to the outside environment by fluidic channels [107]. Two different reagents are fed into each reaction well, where the reaction takes place, followed by spectroscopic detection/identification. This biochip uses nanoliter range reaction volumes and can be fabricated using materials accommodating various chemical conditions. Three-dimensional microfluidic structures for complex integrated processing are currently being developed addressing main applications in both chemical synthesis and genomic screening.

6. Integrated microdevices and other novel applications

Downscaling conventional bioanalytical methods and instrumentation has been one of the central but not the only trend driving the development of the μ-TAS concept. Researchers have therefore focused on exploring ways of building integrated devices, combining separations with pre- and post-processing steps. Amongst the latter are PCR-based DNA amplification, restriction digestion, other enzymatic assays, analyte preconcentration, filtering, dialysis, various post-derivatization and detection methods. Some of these recently reported complex devices have already been mentioned above in the DNA and protein analysis sections. The ultimate integration of all of these steps into a single microchip has not been realized so far, although higher level system integration and complex microdevices are rapidly emerging.

Very recently, the first commercial product has been introduced for chip-based electrophoresis analysis of DNA, RNA and proteins (Agilent 2100 Bioanalyzer) [108]. Another commercial product being developed intends to provide 96-channel microfluidic plastic LabCards [109] for high throughput miniaturized biochemical assays, DNA analysis, etc.,

addressing a whole range of common laboratory procedures such as mixing, incubation, metering, dilution, purification, capture, concentration, injection, separation, and detection. Other types of integrated microchips have been designed and prototyped for clinical applications [45,110,111]. A dual function microchip [45] has integrated two key steps in the analytical procedure – cell isolation and PCR. White blood cells were isolated from whole blood on 3.5 μm feature-sized weir-type filters, etched in silicon-glass 4.5 μl chips, followed by direct PCR amplification of isolated genomic DNA. Modification of filter size and shape and/or using specific capture agents can be employed to increase selectivity of the devices.

A simple elegant method of thermocapillary pumping, based on surface tension changes under localized heating, was incorporated into a microchip structure for guiding nanoliter solution droplets in a controllable manner through the channels, mixing and incubating them to perform enzymatic reactions [110,111]. This integrated system also incorporated thermocycling chambers, microfabricated gel electrophoresis channels and a set of doped-diffusion diode elements, fabricated in Si, for detection of β-radiation (^{32}P-labeled DNA) (Fig. 10). The thermocapil-

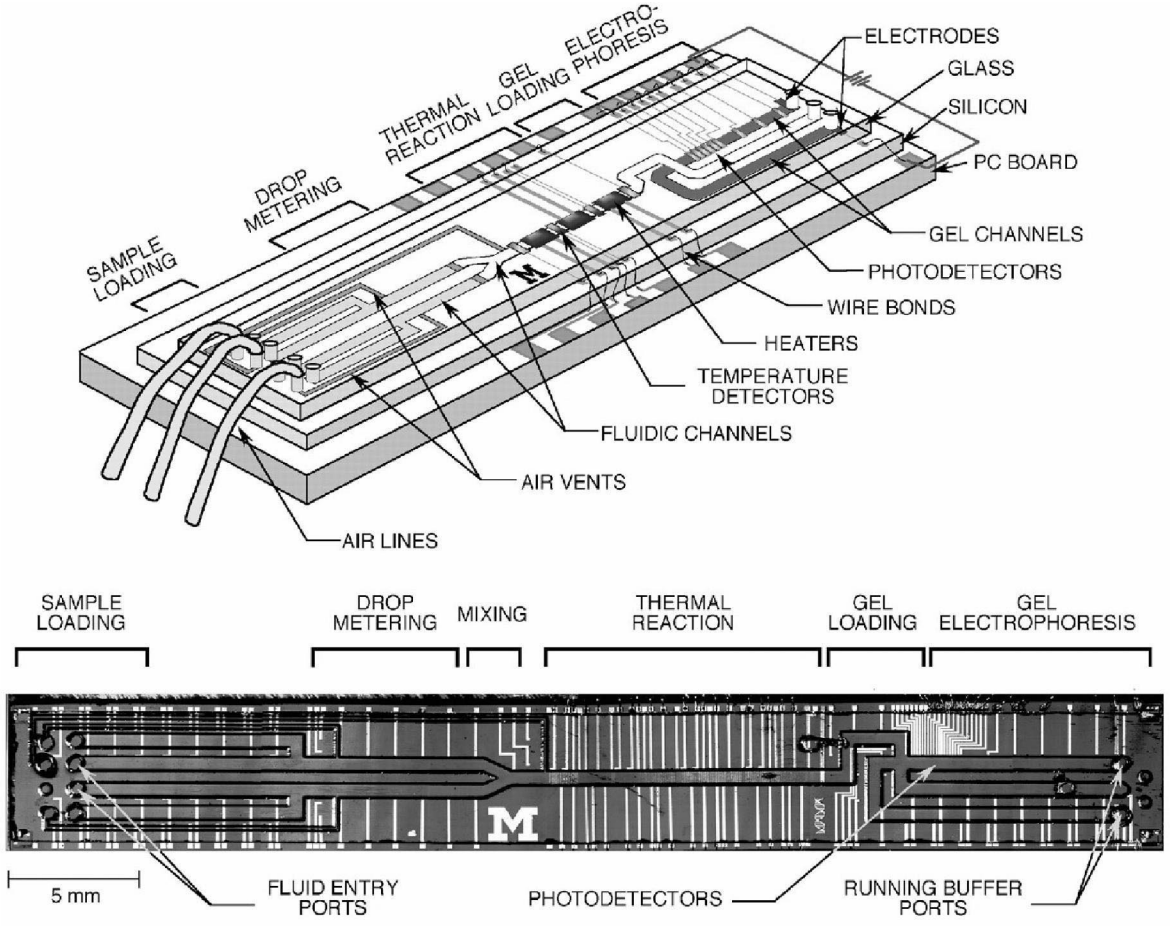

Fig. 10. (Upper panel) Schematics of an integrated device with two liquid samples and electrophoretic separation gel present. The only electronic component not fabricated in the silicon substrate, except for control and data-processing electronics, is an excitation light source, placed above the electrophoresis channel. (Lower panel) Microscopic picture of the device. Wire bonds to the printed circuit board can be seen along the top edge of the device. The pressure manifold and buffer wells, that fit over the entry holes at each end of the device, are not shown. From Ref. [110] with permission.

lary pumping idea was recently also developed by Troian et al., who proposed configurable thermofluidic arrays to pattern flow at the microscale level [112]. The development of this approach will enable ''pixelating'' the chip surface to precisely control the temperature pixel by pixel and, therefore, surface tension and viscosity of the liquid droplets. By this means, it is possible to move, mix and otherwise manipulate small volumes.

An interesting alternative approach to microfluidics combining informatics, bioassays and miniaturization in a ''laboratory on a disc'', has been recently implemented [113]. The so-called LabCD is a consumable compact disc with microscale fluid paths, reaction chambers and valves. Fluids are moved along these pathways by capillary action and centrifugal forces generated by the rotation of the disk, enabling to perform various assay types. Digital information (software) is encoded on an other layer on the same disc, which automatically controls the disc motion and operation and allows recording and analyzing of bioassay data from the microfluidic layer. Microfluidic processes, such as liquid transport, valving, mixing and incubation are accomplished by geometrically designing chambers and interconnecting channels and programming the sequence of the disc rotation. Integrated DNA amplification from blood and bacteria samples was demonstrated by mixing individual components together in the PCR chamber followed by thermocycling [114]. Miniaturized and client-specific integrated CDs are also being developed [115]. These disposable plastic fluidic devices are capable to process hundreds of samples in parallel, combining many common laboratory procedures, such as sample desalting, preconcentration, MALDI target preparation, etc. LabCDs are intended to operate from nl to sub-ml volumes of solutions of biological fluids (e.g., whole blood, urine), which would make them excellent practical diagnostic tools for point-of-care applications, such as doctors offices, etc.

More recently, a flexible elastomer chip has been introduced, fabricated by multilayer soft lithography [116] and incorporating arrays of microscale valves and pumps for precise mechanical pumping and switching of minute quantities of fluids. This technology is based on the studies of Fu et al., which have developed miniaturized devices for cellular and particle manipulation, such as fluorescence-activated flow cytometer [117], representing the first demonstration of cell sorting in microfabricated fluidic channels based on fluorescent properties of biological entities. These disposable sorters were fabricated of flexible elastomers using soft lithography techniques. The cells or beads of different colors were spatially confined in a narrow (3 μm) T-shaped junction manifold, eliminating the need of hydrodynamic focusing used in conventional flow cytometers. The cells were passing through the detection point (probing laser beam) one by one and sorted by simply redirecting the electrokinetic flow into one of the two output channels. Similar microfabricated silicone structures have been used by the same research group for sizing and sorting single DNA molecules in 2–200 kbp range [118] (Fig. 11) allowing absolute measurement of the length of the molecule and eliminating the need for a sizing standard. Unlike in conventional gel electrophoresis, the resolution of single-molecule DNA sizing devices increases as DNA molecules become longer due to improved signal-to-noise ratio. In addition, the method does not depend on analyte mobility, it is much faster than pulse-field gel electrophoresis, and requires a million times less sample. Combining multilayer soft lithography technology with the unique properties of elastomer materials, mechanical valves and pumps were microfabricated [119], that demonstrated precise fluid control over a wide range of conditions. These valves were very fast (ms response level), compact (could be as small as 20× 20 μm) and pneumatically actuated. Peristaltic pumps were implemented by arranging three consequtive valves in a row. The valves and pumps had negligible dead volume, could transport fluids up to a few nl/s, and represented a strong driving force (vs. electrokinetics) to microfluidics. The technology is currently being developed and expected to find applications in a variety of life science areas, including molecular screening and drug discovery.

A filter chamber array, microfabricated in silicon and sealed with glass, enabled real-time parallel analysis of three different samples on beads in a volume of 3 nl on a 1 cm^2 chip [120]. Each filter chamber contained microfabricated pillars to trap and localize the reacting particles. Single nucleotide polymorphism (SNP) analysis by solid-phase

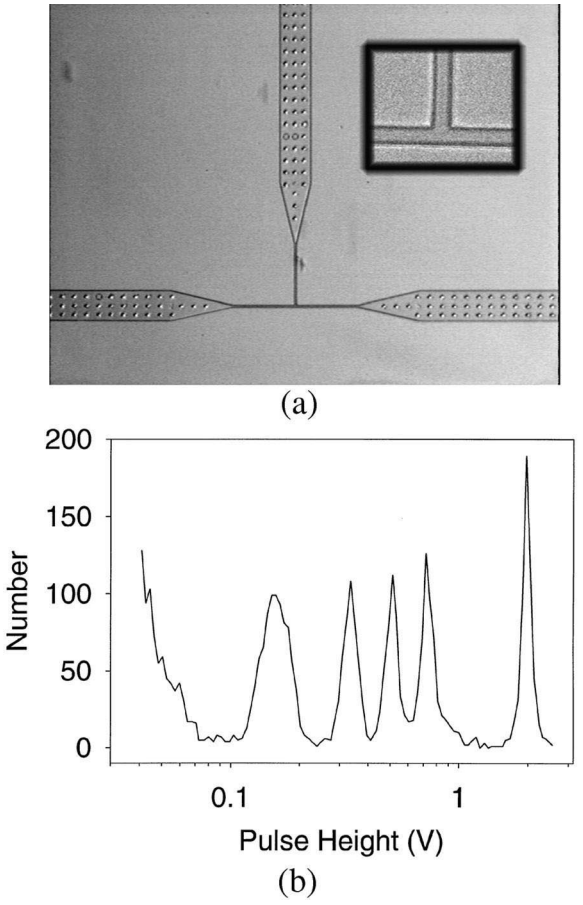

Fig. 11. (a) Picture of a T-channel device. The large channels with lateral dimensions of 100 μm narrow down to 5 μm at the T junction. The channels depth is 3 μm. Support pillars in the wide channels prevent bowing of the elastomer material. The inset shows a magnified view of the T junction. (b) Separation of *Hin*d III digest of λ DNA. Peaks (from right to left): 23, 9, 6, 4 kbp, and the unresolved combination of 2 and 2.3 kbp. From Ref. [118] with permission.

pyrosequencing has been performed in this chamber, using biotinylated primers attached to the streptavidin-coated beads. Allele specific pyrosequencing is based on the difference in DNA polymerase extension reaction of match and mismatch primers hybridized to the target DNA. The nucleotide incorporation resulted in the release of pyrophosphate, which was enzymatically converted to ATP, and detected by luciferase-generated light. Passive fluidic valves, consisted of hydrophobic patches of plasma-deposited octafluorocyclobutane, were incorporated in the device and served as physical barriers between liquids, to allow controllable sequential loading of different samples without their interference. The device is reusable, enables parallel sample handling, and amenable to the implementation of complex biochemical assays on beads (Fig. 12).

A number of studies have been focused lately on using optical forces for manipulation of small objects (cells, bacteria, beads, microdroplets, etc.) in liquids. Known as optical tweezers, or laser trap, this technique allows to select, trap and guide single cells or microorganisms. A new microsystem, presented by Reichle et al. [121], is based on the combination of optical and chip-based dielectrophoretical trapping of cells and beads with micrometer precision. A bead was caught by the laser tweezers and brought into contact with a single cell held in a dielectrophoretic field cage (Fig. 13). The latter was constructed with eight specially arranged microfabricated electrodes, which generated a high-frequency electric (a.c.) field. These manipulations enabled direct cell handling to study intercell ligand–receptor interactions and to conduct measurements on a single cell level. Another microfluidic system [122] has been designed for high speed separation/isolation of a single living cell or microorganism in the presence a large number of other cells in the solution. This was achieved by integrating laser trapping and dielectrophoretic forces. Flow velocity within the microchannel was adjusted to balance the optical force on the cell. The target entrapped in the laser focal point can be transported and isolated through the microchannel system. This is a promising technique for screening microbes, pure cultivation of cells, etc. A microreactor system, based on precise optical manipulation of water droplets in oil emulsion, has also been described [123]. Extremely small quantities of samples (down to single DNA molecule level) can be brought into contact by the fusion of two microdroplets induced by the optical pressure of the laser beam.

7. Fantastic plastic

Recent trends of microfabrication using plastic materials have been described in detail [124,125] and summarized in several reviews elsewhere [4,6].

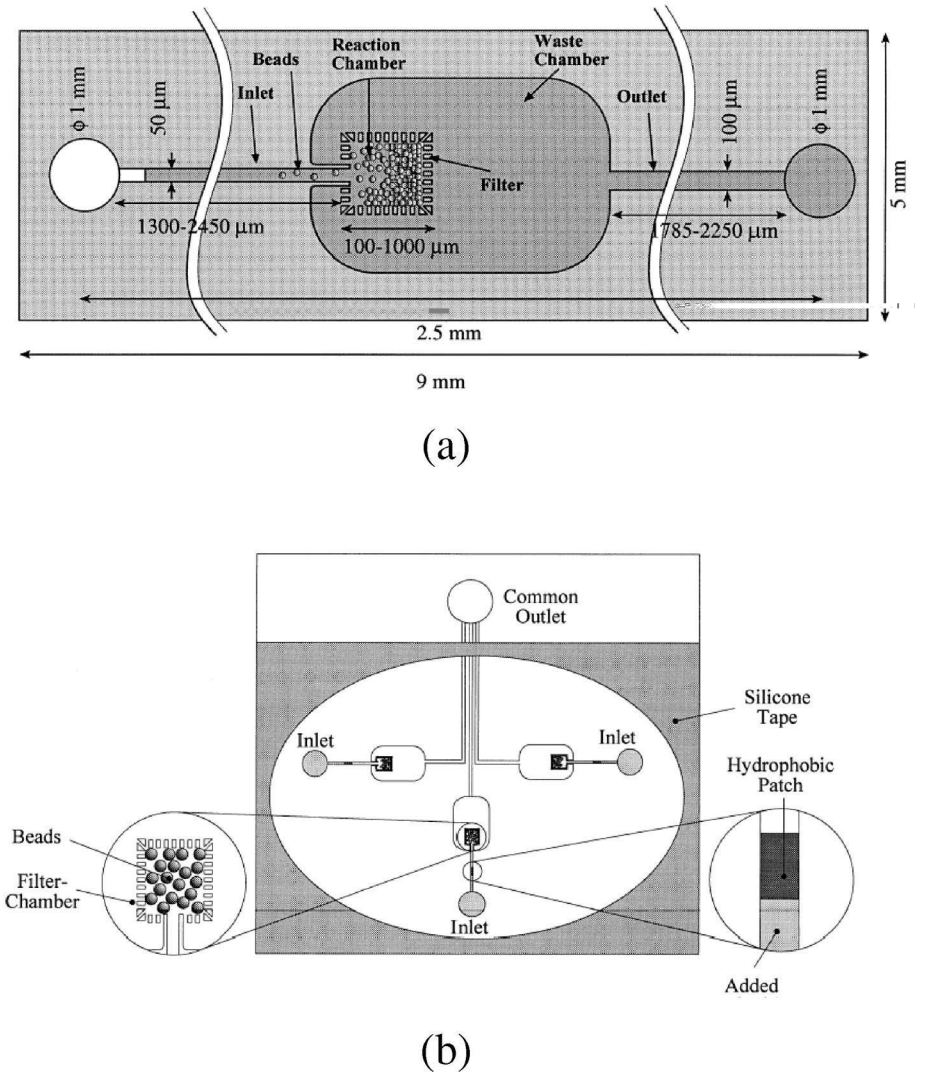

Fig. 12. (a) Schematics of a micromachined single filter chamber device. (b) Conceptual drawing of the filter-chamber array. The two key elements, the filter chamber and the passive valve, are magnified. From Ref. [120] with permission.

Some important advantages of polymeric materials versus more ''traditional'' glass and fused-silica include inert surface characteristics, especially favorable for many biopolymer separations and enzymatic reactions; possibility of high resolution fabrication of deep and shallow features with well-defined vertical walls, for which a number of well developed methods (laser ablation, replication or casting, injection molding, hot embossing, etc.) and a variety of materials (polydimethylsiloxane, polymethylmethacrylate, polystyrene, polycarbonate, cellulose acetate, polyethylene terephthalate, etc.) are readily available; certain chemical resistance; low fabrication temperatures suitable for realization of some delicate biological assays and other on-chip integrations; applicability of fast mass production at low cost. Since we have already mentioned a few interesting plastic-based implementations of microfluidic devices for integrated processing and analysis of biological materials earlier, here we only describe

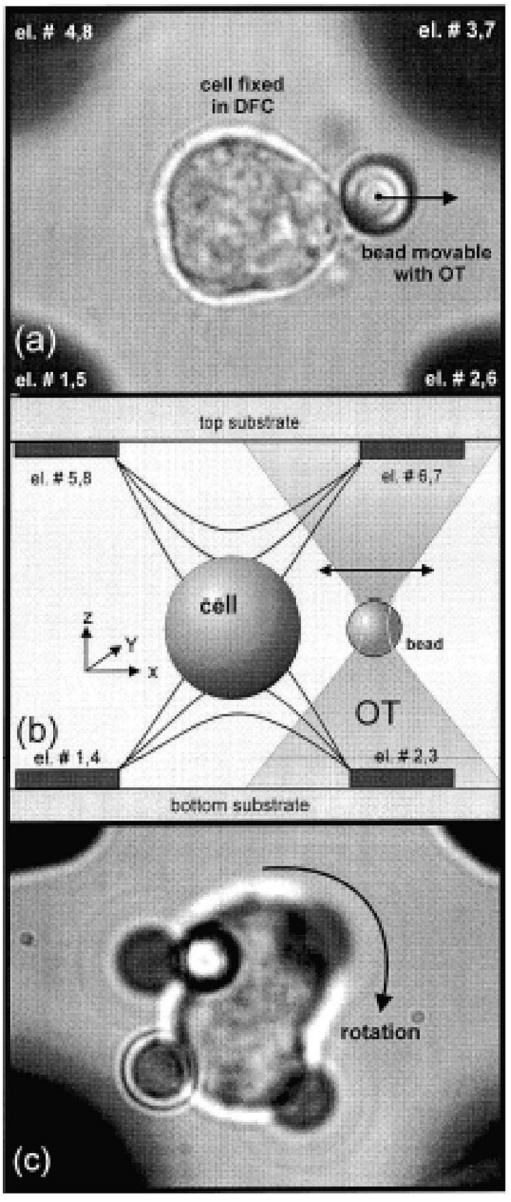

Fig. 13. Combination of dielectrophoretic field cage (DFC) and optical tweezers (OT) for the measurement of bead–cell adhesion: (a) 4.1 μm polystyrene particle trapped with laser tweezers (right) in contact with T-lymphoma cell (~15 μm in diameter). Cell and bead were brought into contact. The time for stable adhesion was measured. (b) Schematic representation of the experimental system used to measure adhesion forces between the bead and the cell with the cell trapped in a DFC and the bead trapped in the laser focus of the OT. (c) Probing different surface regions of the cell for bead–cell adhesion (five beads are attached to a single cell). From Ref. [121] with permission.

some very recent exciting new developments in this area.

The first application of biodegradable polymer microstructures and microdevices was reported by Armani and Liu [126]. The ability to naturally degrade in tissues holds an immense promise for such materials in designing implantable biomedical microdevices. The authors described novel techniques for microfabrication using biodegradable polycaprolactone, which enabled formation of three-dimensional microstructures via silicon micromolding, transferring metal patterns to plastic substrates, and sealing both dry and liquid-filled microcavities with a thin gold film.

A complex poly(methyl methacrylate) (PMMA)-based fluidic device was built by Soper et al. [127], which coupled capillary nanoreactors to microseparation platforms (electrophoresis chips) for the generation of sequencing ladders and PCR products. The nanoreactors consisted of fused-silica capillary tubes with a few tens of nl reaction volume, which can be interfaced with the chips via micromachined (X-ray etching) PMMA connectors. A DNA template was immobilized to the nanoreactor walls via a biotin-strepavidin linkage. Following fast thermocycling in a special chamber oven, the DNA fragments were directly injected (electrokinetically) into the electrophoretic microchip device for analysis. A dual fiber-optic component was micromachined into the plastic chip for integrated laser-induced fluorescence detection, consisting of two channels, accommodating laser light delivery and emission collection fibers. These results are promising in the perspective of fabrication automated devices, which consume minute amounts of costly reagents.

The applicability of a novel and promising polymeric material, Zeonor 1020, to the fabrication of microfluidic chips has been investigated by Kameoka et al. [128]. The authors employed conventional embossing techniques to fabricate electrophoresis microchannel structures coupled with a microsprayer via a microliquid junction. The device provided efficient electrospray MS detection of CE-separated components. Zeonor polymer, commonly used in compact disk manufacturing, is inexpensive and possesses favorable physical characteristics, such as good chemical resistance to organic solvents, ap-

plicability of standard plastic microfabrication techniques, as well as, good bonding and optical properties. All of the above discussed advantages could make this new polymer a promising candidate for future microchip manufacturing.

A very recent series of publications by Locascio and co-workers [68,69,129] had demonstrated successful applications of PDMS, PMMA, copolyester and their combinations for assembling integrated fluidic structures to perform affinity dialysis and sample concentration for fast and sensitive ESI-MS analysis of different compounds. Multilayer devices were assembled from individual plastic pieces imprinted with microchannels by silicon template imprinting and capillary molding techniques. Three-dimensional fluidic devices were constructed by sandwiching a polyvinylidene fluoride membrane between the substrate layers with appropriate channels and simply clamping the assembly together using the good adhesive properties of the polymeric materials. Access holes were drilled in the plastic to interconnect fluidic layers and interface with external inlet/outlet lines. These devices performed affinity capture, pre-concentration, and direct identification of a targeted compound by ESI-MS (coupled to the chip through the capillary and microdialysis junction), as well as miniaturized ultra-filtration of affinity complexes of antibodies [68]. The analyte solutions were pumped through the channel countercurrently to the buffer flow in the adjacent channel (separated by a semi-permeable membrane). By this means it was possible to perform dialysis and separation of aflatoxin affinity complexes with their antibodies from unbound compounds, followed by passing the solution of affinity complexes against an air counterflow in another layer for water evaporation and analyte concentration (Fig. 14). Another similar device performed ultrafiltration of affinity complexes of phenobarbital antibody and barbiturants, including sequential loading, washing and dissociation steps. These microfluidic devices significantly reduced dead volume and sample consumption, and increased MS detection sensitivity by 1–2 orders of magnitude. An integrated platform, based on similar PDMS–membrane assemblies, was presented [69] for rapid and sensitive protein identification by on-line digestion and consequent analysis by ESI-MS, or transient capillary isotachophoresis–capillary zone electrophoresis with MS detection. A miniaturized membrane reactor was fabricated, using a porous membrane with adsorbed trypsin separating two adjacent channels. Due to the large surface-to-volume ratio of the porous membrane structure, extremely high catalytic turnover was achieved. It is worthwhile to mention the last publication of the same group [130], where a microfludic chip has been adapted for a sandwich hybridization assay to detect pathogen bacteria. A PDMS channel was sealed to a gold-coated glass which was used for immobilization of thiolated capture probes and self-assembling layers of blocking liposomes. The reporter probes were tagged with carboxyfluorescein-filled liposomes enabling detection at low target concentrations. The small dimension of the channel and constant flow format enabled to reduce the amounts of reagents and accelerated the assay by apparently eliminating diffusion limitations.

8. Conclusions

The new millennium holds a great promise for the development of microfabricated chemical and biochemical analytical systems. Some of the main advantages of miniaturization are reduced reagent consumption, improved performance, multifunctional possibilities with interconnected network of channels, inherent mechanical stability of monolithic systems, possibility of parallelization and inexpensive mass production. System integration plays one of the most significant roles in miniaturization and will lead to greater process intensification. As miniaturization greatly improves heat and mass transfer, it opens new horizons and leads to revolutionary changes in bioanalytical sciences and separation technology. Microfluidic systems enable rapid identification of nucleic acids, proteins, drugs or other compounds, using sub-nanogram amounts of samples, and provide on-line interface with upstream/downstream processing and analysis, such as sample preparation, microdialysis, separation, microsequencing, cloning, MS detection, etc. The driving force behind the development of microfabricated devices is

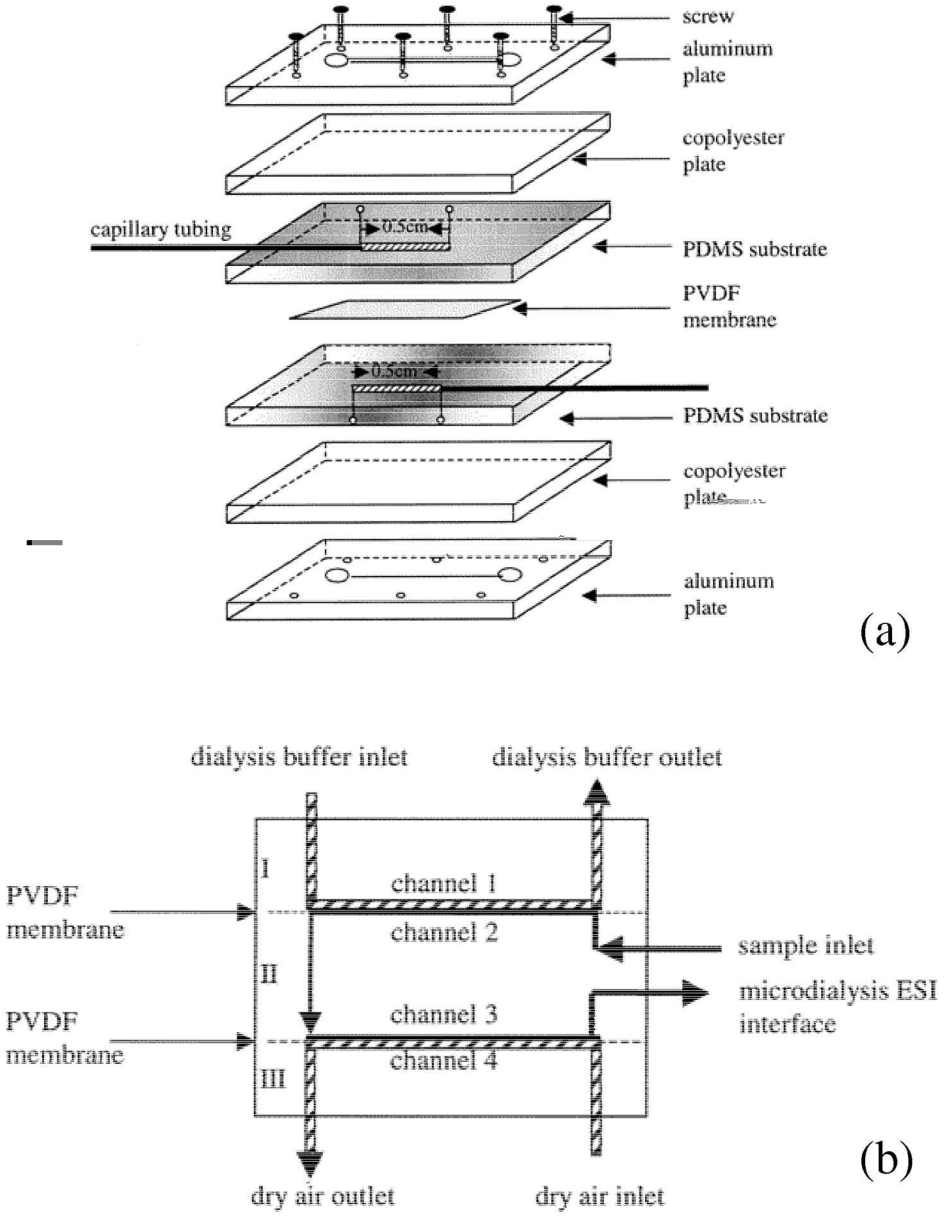

Fig. 14. (a) Assembly of complete miniaturized affinity ultrafiltration system containing preformed PDMS microchannels. Channels are fabricated in PDMS substrates. The copolyester pieces provide structural support to the soft PDMS substrates. (b) Side view schematics of the miniaturized affinity dialysis and concentration system. I–III indicate top, middle and bottom imprinted copolyester pieces, respectively. Piece II is imprinted on both sides. Two poly(vinylidene fluoride) (PVDF) membranes separate the copolyester channels. From Ref. [68] with permission.

certainly the commercialization of microfluidic technology with its tremendous potential of large-scale applications in genomics, proteomics, drug discovery and medical diagnostics. These areas demand disposable analytical microdevices that can be mass manufactured at low cost.

Acknowledgements

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