

Microfabricated reaction and separation systems

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Over the past year there have been a number of recent advances in the fields of miniaturized reaction and separation systems, including the construction of fully integrated 'lab-on-a-chip' systems. Microreactors, which initially targeted DNA-based reactions such as the polymerase chain reaction, are now used in several other chemical and biochemical assays. Miniaturized separation columns are currently employed for analyzing a wide variety of samples including DNA, RNA, proteins and cells. Although significant advances have been made at the component level, the realization of an integrated analysis system still remains at the early stages of development.

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Current Opinion in Biotechnology 2001, 12:92–98

0958-1669/01/\$ – see front matter

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Abbreviations

CE capillary electrophoresis
CL chemiluminescence
EC electrochemical detection

Introduction

Photolithographic microfabrication is a mature technology developed and optimized by the microprocessor industry. Semiconductor microfabrication serves as an excellent platform for developing miniature integrated analysis systems with short analysis times, reduced sample-volume requirements and cost efficiency. These microanalysis devices can be classified into two broad categories based on the complexity of the fluidics involved: microarray-based (DNA or protein immobilized on the chip) and microfluidic-based (DNA or proteins being transported, reacted and separated on the chip) microdevices [1]. In this review, we focus on recent developments in microfluidic systems for the analysis of DNA, proteins and other biomolecules.

There has been a burst of activity in the analysis of biomolecules other than DNA and proteins using microdevices. Biosensors have been developed for the detection and analysis of physiologically relevant molecules, such as glucose, lactic acid and ascorbic acid [2*], and detection of environmental agents and herbicides, such as 2,4-diphenoxyacetic acid [3]. In the area of DNA analysis, reaction volumes range from a few microliters [4*] to a few hundred nanoliters [5],

and although reaction volumes can easily be reduced further, speculation persists on whether this is indeed a viable venture when developing diagnostic devices for the detection of infectious agents. This caution is because of the fact that sample volume statistically limits the number of available targets for the detection in a given assay [6].

In this review, we discuss recent advances in various components of the microfluidic analysis system and also report attempts at constructing integrated microanalysis systems.

Microfabricated reaction systems

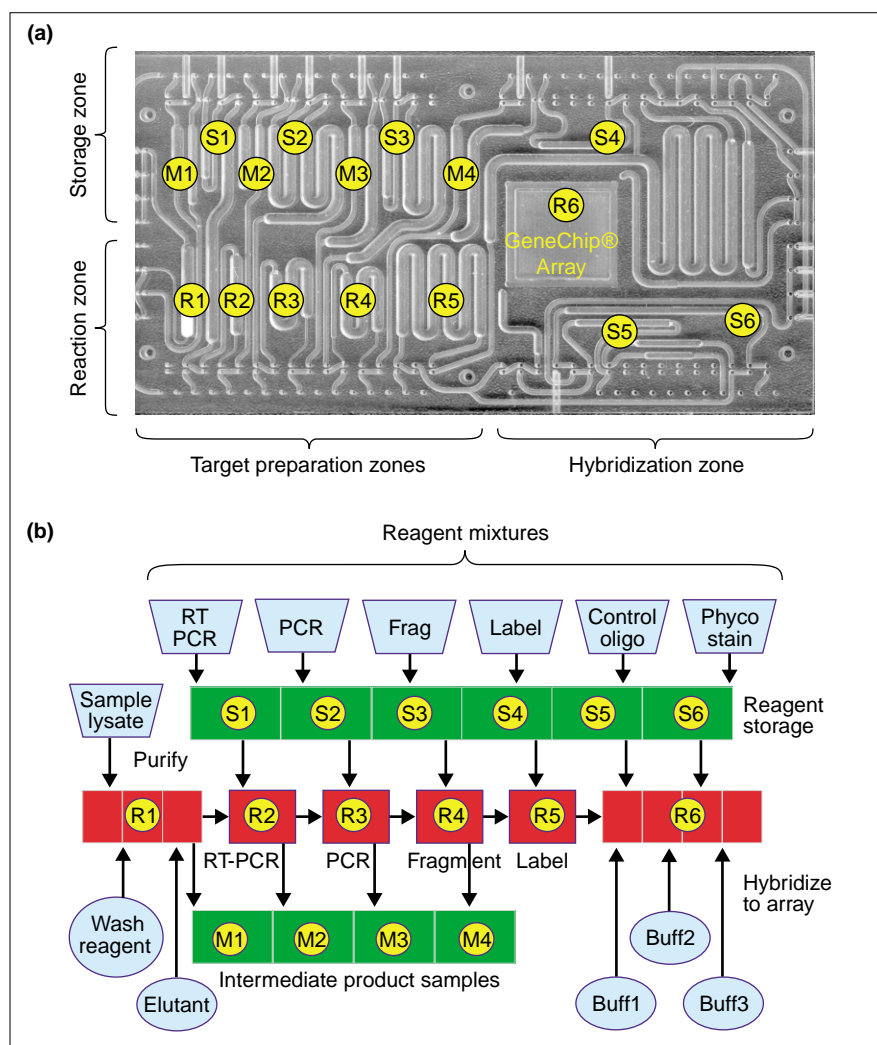
The general trend in the area of microfabricated DNA analysis is toward devices with multiple functions that perform multiple reactions in series and/or parallel. The major operations now performed in DNA analysis devices include cell lysis, sample concentration and enzymatic reactions such as reverse transcription, PCR, DNase digestion and terminal transferase labeling [4*,7] (Figure 1). Several groups also report combined PCR and electrophoretic analysis of reaction products [5,7,8]. Sanger DNA sequencing was performed in a solid-phase nanoreactor directly coupled to capillary gel electrophoresis [9]. With the current interest in performing PCR in a microchip, the choice of substrate material has become an important issue. The trade-off in materials reduces the need for low-thermal conductivity for thermal isolation in a multiple reaction device versus the need for higher conductivity for effective heat removal for rapid cycling in PCR. Polycarbonate and glass devices have been reported for on-chip PCR in conjunction with other reactions [4*] and an electrophoresis module [5,7]. In addition to thermal isolation, a significant challenge in performing PCR in microfabricated devices is associated with controlling the evaporation of the reaction. This problem has been addressed through the use of diaphragm valves [4*].

An important area in DNA analysis is the analysis of gene expression. Gene expression analysis on the microscale has traditionally been performed by hybridization in microarrays. A recent development on the conventional method of passive, non-active hybridization is dynamic hybridization using DNA probes pumped through target-bearing paramagnetic beads [10]. Traditional hybridization systems are not electronically active; Radtkey *et al.* [11*] describe a method for the discrimination of short tandem repeat (STR) alleles based on active microarray hybridization. A rapid analysis of STR alleles using passive hybridization techniques (required in non-active microarrays) is currently very difficult.

In the area of protein analysis in microchips, a device has been demonstrated that performs enzymatic reactions,

Figure 1

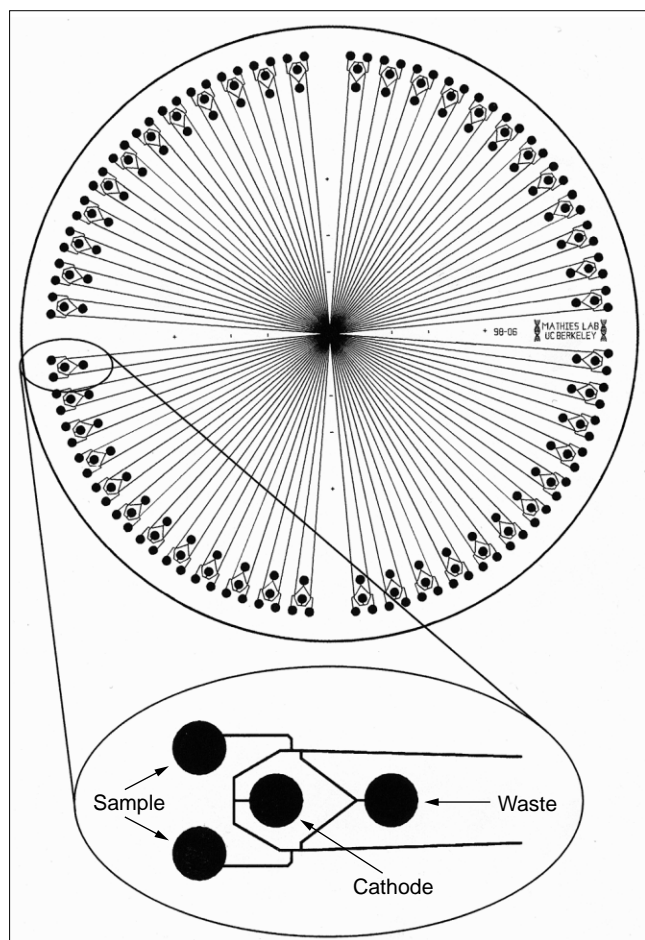
(a) An integrated device that automatically performs a multi-step HIV genotyping assay. (b) The steps include RNA extraction, RT-PCR, nested PCR, DNase fragmentation and dephosphorylation, terminal transferase labeling, dilution and hybridization, washing, phycoerythrin staining and washing. S1–S6 are the reagent storage chambers, R1–R6 are the reaction chambers and M1–M4 are the intermediate product storage chambers. Reproduced from [4*] with permission.



electrophoretic separation of the reactants from the products, and post-separation labeling of proteins and peptides prior to detection [12]. In this work, the authors performed tryptic digestion of the insulin B chain and reduction of the disulfide bridges of insulin on a microchip [12]. Another area that appears to have benefited significantly from increasing interest in the development of microanalysis devices for a wide variety of biomolecules has been the area of immunoassays. Immunoassays typically require very high specificity and are time-consuming and expensive. Reports on chip-based immunoassays usually focus on separation of the free form of the antigen from the antigen-antibody complex by capillary electrophoresis (CE); there have been few reports on the antigen-antibody reaction performed on-chip [13]. In a recent report on a microdevice-based immunosorbent assay, detection of secretory human immunoglobulin A was performed on polystyrene beads in a microchip [14]. The time required for the assay was reduced by two orders of magnitude compared to the traditional assay in microtiter plates.

In clinical diagnostics, although they return chemical information rapidly, microanalysis devices have limits in versatility and scope because of minimal sample-handling capability (associated with their limited surface chemistry). Notwithstanding, there have been reports in the literature on bioassays for clinically relevant molecules. One such report is a reaction/electrophoresis chip for simultaneous bioassays of glucose, uric acid, ascorbic acid and acetaminophen [2*]. A binding assay for biotin has been reported in a microfabricated picoliter vial [15]. Results indicate that detection limits of the order of 10^{-14} mol of biotin are possible. These binding assays based on picoliter volumes have potential applications in a variety of fields including microanalysis and single-cell analysis (where the amount of sample is limited) as well as in high-throughput screening of biopharmaceuticals. Along these lines, from the perspective of environmental microdevices, the sensing of biological substances based on the bending of microfabricated cantilevers has been demonstrated in the detection of the herbicide 2,4-dichlorophenoxyacetic

Figure 2



Schematic of a 96-channel radial capillary electrophoresis microplate. The device has separation channels (100 μm wide and 50 μm deep) with 200 μm twin-T injectors. Detection is performed using a laser-excited galvoscaner. In this system, 96 different DNA samples can be injected, separated and detected in less than 8 min. Reproduced from [32**] with permission.

acid (2,4-D) [3]. The cantilevers were coated with 2,4-D and the deflection was measured while continuously rinsing with a solution containing monoclonal antibody.

On another front, there has been significant activity in the recent past in the area of culturing cells and tissues in microfabricated devices. An important and interesting advance in cell culture in microdevices is the neurochip, a silicon micro-machined multielectrode device upon which cultured mammalian neurons can be continuously and individually monitored and stimulated. The neurochip significantly improves on earlier methods by associating individual electrodes with the cell bodies of each of the neurons in a small network [16**]. The neurochip is based on a 4×4 array of metal electrodes, each of which has a caged well structure designed to hold a single mature cell body while permitting normal outgrowth of neural processes. The device is capable of maintaining cell survival, and the electrodes can both record and stimulate electrical activity with no crosstalk between

channels. In addition, surface microfabrication techniques have been widely used for the spatial control of cells in culture. Many strategies have employed variations in surface charge, hydrophilicity and topology to regulate cell functions such as attachment. Ito has published a good review in this area [17].

Separation and detection systems

Microseparations

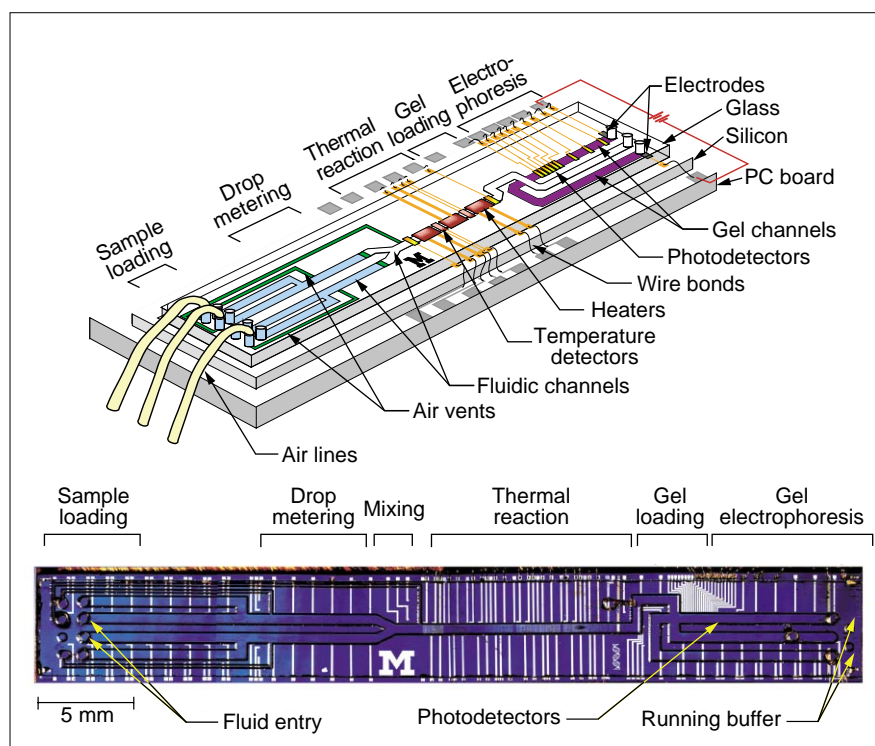
Microfabricated separation systems have become integral components for chemical analysis after the critical evaluation of the benefits of miniaturization was presented by Manz *et al.* in 1993 [18]. A large number of articles published in recent years offer insights into fabrication procedures, choice of materials, design considerations and mode of operation of microchips for separations applied to a wide variety of analytes [19–22,23*]. DNA continues to be the main molecule of interest; other molecules being studied include RNA, proteins and peptides. A noticeable trend in microseparations has been the dominance of electrophoretic techniques over chromatographic techniques. This trend is predominantly because pumping liquids in chromatographic separation systems requires more engineering effort than electrophoretic systems; electrokinetic systems can be operated by simply changing the applied voltages. Another significant trend is the constant push towards improving separation power (higher resolution, faster analysis times) while keeping the separation lengths short.

One principal area of interest is improving the quality of separation by altering the type, composition and the quality of sieving media. A wide variety of existing polymers and novel materials are being investigated to achieve enhanced resolution and quality of separation [24–29]. For DNA separations, both crosslinked and non-crosslinked gels are being investigated. Crosslinked gels offer the advantage of shorter separation lengths and lower applied voltages but suffer from nonreusability [28,29]. Non-crosslinked gels operate under relatively high voltages but offer the desirable feature that the sieving matrices can be exchanged after each run [24–27]. Single base pair resolution up to 800 bases using denaturing linear polyacrylamide solutions run under optimized conditions has been reported [30*]. Capillary array electrophoresis for massive parallelization of electrophoresis has also been demonstrated [31,32**] (Figure 2).

Separation resolution also depends on the sample injection and applied voltage. Several types of injection geometries including simple T, cross and double T configurations have been reported for sequencing DNA [33]. Microfabricated porous membrane structures have been constructed to concentrate DNA samples before injection [34*]. High voltages required for running non-crosslinked CE systems can easily be attained by microfabricating metal electrodes within the microchannel. Many researchers are investigating the most optimal conditions to obtain high speed/resolution sequencing; several papers provide fundamental insights on band dispersion and suggest ways to reduce band broadening [35–37].

Figure 3

Schematic and photograph of an integrated device with a nanoliter liquid injector, a sample mixing and positioning system, a temperature-controlled reaction chamber, an electrophoretic separation system and fluorescence detectors. The device is capable of measuring aqueous reagents and DNA-containing solutions, mixing the solutions together, amplifying or digesting the DNA to form discrete products and separating and detecting those products. Reproduced from [61] with permission.



Advances in microfabrication and nanofabrication techniques have opened doors for novel ways of separating biomolecules. Cell sorting has been demonstrated in microfabricated arrays [38]. Long DNA molecules have been separated in microfabricated asymmetric obstacle courses [39••] and entropic trap arrays [40••]. Single molecule sizing and sorting devices made out of silicone elastomer have been developed [41]. Sodium dodecyl sulfate (SDS) capillary gel electrophoresis of proteins has been demonstrated in planar microchannels resulting in faster separations while retaining macroscale resolution [42]. A microfluidic diffusion-based separation system has been reported for extracting small molecules from blood [43••]. In the future we will probably witness DNA sequencing and oligonucleotide separations performed in novel, custom-made media constructed from materials such as carbon nanotubes. Also, microseparation devices made out of plastic substrates by simple injection molding and/or hot embossing techniques will become more widespread and will reduce fabrication costs considerably [44].

Detection

The power of a miniaturized chemical analysis system is ultimately limited by the ability to detect low concentrations of the analyte. Microseparation systems currently rely on one of the three major detection modes: fluorescence, electrochemical (EC) detection or chemiluminescence (CL) detection [45]. Fluorescence detection is an accurate, time-tested technique, and the dyes currently used are extremely sensitive to the analytes, permitting low concentration detection in femtoliter samples. The primary method of this

detection system for DNA detection is laser-induced fluorescence, which can detect single molecules of DNA on CE chips [46•]. Improvements have been made by introducing optical fiber liquid core waveguides [47–49]; however, fluorescence detection requires a large and expensive supporting optical system that minimizes the advantage of cost and portability. Development of on-chip fluorescence detectors has been reported and will aid in the realization of integrated ‘lab-on-a-chip’ systems [28].

EC detection offers considerable promise for detection in micromachined chips because of the remarkable sensitivity, tunable selectivity and low volume required. CE chips with integrated EC detectors were made in 1998 [50]. Subsequently, Wang *et al.* [51] reported a different microchip CE-EC system with thick-film EC detectors that provided lower detection limits than those previously reported. Detection of single-stranded DNA by using polymer-modified electrodes has also been demonstrated [52]. Recently, Martin *et al.* [53] reported a CE-EC microchip, fabricated in polydimethylsiloxane that employs dual electrodes for detection.

CL detection has been widely used for the analysis of metal ions and immunoassays, because no light source is required in CL measurements and the instruments for CL are much simpler than those for optical methods. This method has the potential to be integrated onto a chip for analyte detection [54]. Hostis *et al.* [55] reported an electrochemiluminescence (ECL) detector and a microenzymatic reactor combining

silicon and polymer technologies. The use of CL is not widespread; more research is needed to produce robust and/or universal probes to increase the technique's applicability.

Integration

The extraction of useful analytical information, such as DNA sequence, from a sample involves a series of chemical manipulations. These manipulations include metering and mixing of reagents, thermal cycling, labeling and fragment analysis. An integrated miniaturized chemical analysis device is a system capable of performing all of the above operations in microscale and nanoscale volumes. Microfluidic components such as channels, valves, and pumps form the core of integrated devices. A wide variety of micromachined valves and pumps have been reported [56,57]; however, to date, none of them have been integrated onto complex analysis systems involving reaction and separation components. The key solution to integration is to have robust valves and pumps that involve simple fabrication procedures and (preferably) do not involve moving parts [58]. Electro-osmotic pumps have found wide acceptance in separation devices mainly for this reason [59].

Though the power of integration is widely appreciated [60], very little progress towards constructing integrated devices has been reported [61,62]; however, several functional integrated devices have been reported that employ macroscale assembly of components as opposed to fabricating all the components on the same platform [7,8,12]. Also, there have been very few published reports on sample preparation, a potentially important area for integrated microdevices (Figure 3).

Conclusions

In the future we shall witness advances at the component level: higher resolution separation systems, smaller volume reaction chambers, and ultrasensitive detectors. Novel materials will be used for fabrication: plastic microchannels, nanostructured sieving matrices, and polymer-based light emitting diodes (LEDs) and detectors. Trends in commercialization show the push towards plastic substrates that can be processed by cheaper fabrication techniques like injection moulding, hot embossing, and casting [63,64–66]; however, conventional photolithographic machining of silicon and glass will still have a place in several applications, particularly where active on-chip electronics is essential.

To actually accomplish genetic or other biochemical analysis, individual micromachined components are more powerful when linked together to function as an integrated device; this will continue to be the focus of many research groups. Component development without some integration will not find widespread commercial applications of microchips. However, not all components need to be integrated for all applications.

The greatest impact of these integrated microchips will most likely be in the area of personalized healthcare involving the analysis of infectious diseases, the identification of

genetic predisposition to diseases, and treatment of the same using custom-designed drugs. Personal identification based on DNA sequence will eventually be accomplished using microchip scanners. Portable chemical analysis devices will find applications in forensics, agriculture, and the exploration of space. In the not too distant future, microfabricated chemical reaction and separation systems will be as prevalent as microprocessors are today.

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