Future trends in diagnosis using laboratory-on-a-chip technologies

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SUMMARY

There has been an enormous growth in the development of biotechnological applications, where advances in the techniques of microelectronic fabrication and the technologies of miniaturization and integration in semiconductor industries are being applied to the production of Laboratory-on-a-Chip devices. The aim of this development is to create devices that will perform the same processes that are currently carried out in the laboratory in reduced timescales, at a lower cost, requiring less reagents, and with a greater resolution of detection and specificity. The expectations of this Laboratory-on-a-Chip revolution is that this technology will facilitate rapid advances in gene discovery, genetic mapping and gene expression with broader applications ranging from infectious diseases and cancer diagnostics to food quality and environmental testing. A review of the current state of development in this field reveals the scale of the ongoing revolution and serves to highlight the advances that can be perceived in the development of Laboratory-on-a-Chip technologies. Since miniaturization can be applied to such a wide range of laboratory processes, some of the sub-units that can be used as building blocks in these devices are described, with a brief description of some of the fabrication processes that can be used to create them.

Keywords: Biotechnology, laboratory-on-a-chip, microfabrication, microfluidics, electrokinetics.

INTRODUCTION

With the increasing number of complex analytical assays being carried out every day in diagnostic laboratories there is an obvious advantage of improving test throughput, analysis time and overall costs of the test. An evolving technology that addresses these issues in that of laboratory-on-a-chip devices. These new devices, most of which are in academic or commercial research and development stages, have their origins in the development of diagnostic industries and microelectronic fabrication. For many years, the microelectronics industry has made use of semiconductor materials such as silicon to produce very large-scale integrated circuits, which contain millions of transistors per square centimetre. Over the many years spent developing new integrated circuits, a wide range of fabrication techniques included etching, deposition of materials, interconnections and packaging have been developed which allow billions of devices to be reliably manufactured every year. Similarly, in chemistry and biotechnology, there is a move towards the development of technologies to enable high-throughput screening. For instance when searching for potential targets in drug development extensive potential compounds will need to be tested. Some companies have assembled chemical 'libraries' of hundreds of thousands of compounds, many of which consist of no more than trace amounts. New methods have to be derived for the rapid inspection of their biochemical activity. Also, in biotechnology the analysis of DNA requires many base combinations to be analysed. Such analysis has the obvious parallel with large-scale integration with the aim of a single unit capable of performing a complete analysis in one simple automated procedure.

The aim of laboratory-on-a-chip technology is to miniaturise current processes to allow them to be achieved in a small self-contained device usually less than 5 cm^2 in size. There are many advantages in the fabrication of such devices. The miniaturization of a process will lead, by definition, to a reduction in the quantity of reagents required for the process, which in turn leads to a reduction in the cost for each analysis or assay. Since the volumes required in a miniaturized process are also much less than conventional laboratory equipment, the speed at which a process can be performed can be increased. For instance a diffusion-limited reaction will take much less time to occur to completion in a small volume than a large one. When coupled with the ability to incorporate automation in the design and handling of chip-based assay systems, considerable time can be saved by miniaturization. As more diagnostic tests are developed using this technology it will become possible to standardize the testing of infectious diseases. This will have important public health implications in the tracing, monitoring and therapy of human disease, and as new devices are introduced in environmental, food, livestock and agricultural testing. Larger samples of tests will be able to be compared from any laboratory worldwide, whilst advances in the availability of information on the Internet allows for information databases to be updated, accessed and assessed by larger numbers of

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people from laboratories worldwide. The Genetic Analysis Technology Consortium (GATC) has been formed by a number of the key providers of chip-based diagnostic tools in the hope of establishing such industry-wide standards, created to provide a unified technology platform to design, process, read and analyse DNA-arrays.

The manufacturing precision of chip-based systems allows for high degrees of control on experimental conditions. Each chip is identical in the dimensions of fluidic channels, position of reaction vessels, heaters, sensors etc. as well as the materials with which it is constructed. Such excellent duplication of experimental conditions aids in laboratory quality control and experimental reproducibility, whilst allowing better transportability of experiments to other environments e.g. from a laboratory bench to a field test. An additional advantage of miniaturization is the ability to apply massive parallelism to experiments by using many chip-based devices at the same time without the need for large amounts of laboratory space and staff. Exploiting devices in parallel further improves throughput and helps reduce assay costs.

This review is aimed at introducing the topic of laboratory-on-a-chip technology. As mentioned above, the subject is an evolving one with much of the work still in the development phases. However, the advantages of chip based devices are many and their application vast. Here we propose to introduce the 3 main areas of biology where chip-based devices are currently being aimed, DNA analysis, biochemical analysis and bioparticle analysis, each of which has significance in diagnostics. In addition to this we will discuss the approaches that a number of commercial entities are taking to produce practical chip-based devices for these markets. Since miniaturization can be applied to such a wide range of processes, we will also discuss some of the practical sub-units, which can be used as the building blocks for new laboratory-on-a-chip devices. These include familiar laboratory tools such as pumps, filters, mixers, heaters and detectors. For further information, we have included a very brief description of some of the fabrication processes that are used to produce a wide range of miniaturized devices. It is hoped that knowledge of the fabrication issues will stimulate new ideas for device sub-units and hence further applications of laboratory-on-a-chip devices. An example of this is the new directions the common 96-well plate are beginning to take, which will also be discussed.

LAB-ON-A-CHIP STATE OF THE ART – CASE STUDIES

There has been, in the last 5 years, an enormous growth in the number of new biotechnology companies exploiting advances in the fields of genetics (Persidis, 1998). Similarly, microelectronics industries have seen massive growth, heralded by developments in miniaturization and integration in semiconductor industries. Technology advances have also been applied to the use of light on microchips, giving birth to optoelectronics industries and applications ranging from the CD-ROM to fibre-optic telecommunications. The technological goal in the development of laboratory-on-a-chip technology is to employ existing expertise in microelectronics, optoelectronics and microfabrication to improve the speed of diagnosis by decreasing the size of diagnostic devices. Smaller devices, which require less reagents, will perform the same processes that are currently carried out in the laboratory on a large scale in less time, with a greater resolution of detection and specificity and at a lower cost. In 1998, *Caenorhabditis elegans* was added to the list of infectious and other organisms whose genomes have been mapped. The desire to complete the mapping of the human genome has, for example, demonstrated the need to develop new tools to provide the molecular biologist with more complex systems to carry out faster analysis incorporating a large degree of parallelism. The expectations of the laboratory-on-a-chip revolution is that technology will facilitate the rapid advances in gene discovery, genetic mapping, gene expression monitoring and in the understanding of human genetic variation (Mastrangelo, Burns & Burke, 1998). Broader application areas to which these technologies are being applied range from infectious diseases and cancer diagnostics, food quality testing and environmental testing. A review of the state of development in the major players in this field reveals the scale of the ongoing revolution and serves to highlight the advances that can be perceived in the development of lab-on-a-chip technologies.

Companies such as Nanogen and Affymetrix are making major advances in the application of chip technologies to the fields of DNA analysis. Nanogen has focused on DNA diagnostics using a combination of electrophoresis, long DNA probes and imaging techniques. This technology comprises an electronically-addressable silicon microchip that can be loaded with DNA capture probes using electrophoretic forces to bind the probes to specific sites on a chip array. This array technology enables Nanogen to custom build their chips with specific probes of relevance to the test sample. There are several advantages that such a chip-based assay offers clinical diagnostics. These include the ability to simultaneously perform many tests on the same sample and a dramatic reduction in the detection time. Concentration of test samples at the test sites allows for rapid hybridization with the captured DNA probes using electric fields. Recent published work has demonstrated the concept of isolating carcinoma cells form peripheral blood (Cheng *et al.*

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1998*a*) and analysing the DNA/RNA from *E. coli* on microfabricated bioelectronic chips (Cheng *et al.* 1998*b*).

The use of long single-stranded DNA fragments enables Nanogen to increase the certainty of identification by preventing mismatch thus ensuring that bound pairs are truly complimentary. The current chip design contains a 5×5 array of 25 electrodes in an active area of 1 mm^2, however, the company is planning to develop larger arrays with up to 10000 test sites. Analysis of the binding of probes on the chip is achieved by using a combination of fluorescent detection and a mixture of pattern recognition and opto-electronics to enable the array to be scanned in minutes at a detection level of hundreds of molecules per test site. The feasibility of engineering such high density electrically addressable microstructures is a result of exploiting advances in the semiconductor industry that have enabled the microprocessor to contain millions of switches on a single silicon chip. The combination of this complementary metal oxide semiconductor technology (CMOS), used to produce integrated circuits, with the miniaturization of electrophoresis and fluorescent detection systems, that are in wide use in diagnostic laboratories, will allow Nanogen to further increase the complexity of the microchips. Nanogen has established corporate alliances to exploit infectious disease diagnostics markets with Becton Dickinson and Company, drug discovery with Hoechst AG and genomics with Elan Corporation, plc. as part of its strategy to expand the applications and accelerate the commercialization of products derived from its technology.

Affymetrix is another company that has been producing operational GeneChips to identify mutations associated with drug resistance in HIV patients since 1996. This technology is presently being used by Glaxo Wellcome to build a preliminary database of genetic and clinical information of patients with HIV. It has formed collaborations with a number of industrial partners to develop technological aspects of manufacturing the optical chip scanners (Hewlett-Packard) and pharmaceutical companies to test custom probe arrays for genes specifically selected by the partners (F Hoffman-LaRoche). Links with companies in the clinical diagnostics area (BioMérieux) have also been made to develop the arrays for bacterial identification and antibiotic resistance analysis.

The GeneChip technology consists of miniaturized, disposable high-density arrays of DNA probe. The heart of the technology relies on the ability to use a light-directed chemical synthesis process to construct the multi-probe arrays simultaneously on a large glass wafer. Photolithographic processes common to semiconductor industries are used to bind the DNA probes to the GeneChip array surface. The synthesis process involves selectively illuminating the array by passing light through a photolithographic mask. Areas on the chip surface exposed by the light become activated. A chemical reaction follows with the DNA building blocks being formed by DNA coupling during incubation. A new mask pattern can be applied to expose new areas of the array. With repeated cycles of selective activation of the surface in predetermined patterns, many different DNA probes can be built up in a small number of steps until the desired set of probes is obtained.

This synthesis process occurs in parallel with a number of arrays being formed on a single wafer. The wafer is diced and the arrays packaged in chambers ready for hybridization. Such production techniques, familiar to integrated circuit manufacturers, produces large economies of scale in the manufacture processes and enhances the reproducibility of the arrays to be maximized. The GeneChip can now be allowed to hybridise with the sample nucleic acids. Hybridization is detected using fluorescent markers. These markers are used as reporter groups and the position of the matches is identified using a scanner that monitors the patterns of illumination and compares the complimentary pairs with the known position of probes on the arrays.

Affymetrix current markets a range of GeneChip devices aimed at the clinical diagnostics markets. The p53 GeneChip detects single nucleotide polymorphisms of the *p53* tumour-suppresser gene that are thought to be contributors to human cancers. The test will enable the clinician to make an early diagnosis and choose the most effective form of therapy for the particular mutation. The HIV GeneChip enables mutations that are associated with drug resistance to be identified, again helping the clinician to choose the best form of combination drug therapy for the individual patient. Other chips are being developed to detect the breast cancer gene, *BRCA1*, as well as bacterial pathogens.

The revolution in semiconductor fabrication has laid the foundations for a revolution in clinical diagnostics. A further reduction in the feature size of the GeneChip array will lead to significant savings in time and cost associated with diagnostic tests. The initial 20000 DNA probes that were held on the first chips have been increased to 400000 in the system developed by Affymetrix with Hewlett-Packard. The feasibility of adding such complexity to the biochip technology has greatly excited companies involved in drug discovery and development, and allowed researchers extensive insights into gene discovery and the genetic function. Companies such as Incyte and Synteni, who joined forces in 1998, have developed their own micro-arrays carrying cDNA probes on chips called GEM™ (Gene Expression Microarray) chips. These custom built chips are currently being used in human, animal, microbial and plant

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genomics for drug development, and to create genomic databases. Each array can contain 10000 unique gene sequences with a 500 to 5000 bp length.

Laboratory-on-a-chip technology has also been applied to chemical and biochemical analysis. Companies such as Cepheid, have combined microfluidics and microelectronics to develop integrated systems for fast, accurate detection of infectious diseases, human genes, and industrial and environmental contamination. The microDiagnostics™ system, the result of a marriage of micro-instrumentation and micro-machining, takes advantage of temperature-controlled reaction sites, low cost fluorescence detection and micro-fluidics. Its micro-fluidics technology is capable of accepting large volume, biological fluid samples, to automatically extract, purify, concentrate, mix and present the final mixture for PCR amplification. The core technology relies on the miniaturization of the widely used PCR process with their proprietary I-CORE™ (intelligent cooling/heating optical reaction) technology for rapid thermal cycling on solid state components. An advantage of PCR on the micro scale is that the reaction products can be thermally cycled at the much higher rates of minutes rather than hours. This faster thermal cycling process helps to minimize side reactions that occur at intermediate temperatures causing poor product specificity. Microfluidic integrated components are used to allow automated sample preparation and processing with custom built micromachined silicon structures to capture and concentrate DNA.

Caliper Technologies has been developing analytical testing technologies to meet the specific needs of analytical chemistry or clinical diagnostics laboratories by applying advanced engineering and microfabrication technology to the development of liquid integrated circuits. The same processes of miniaturization and integration developed in semiconductor fabrication are being used to produce microscopic versions of liquid handling and biochemical processing devices such as pumps, valves, volume measuring devices, reactors, extractors and sophisticated separation systems. These devices, called LabChip™, are then linked one after another to create complete chemical processing systems creating a miniature laboratory-on-a-chip. Most laboratory procedures have remained cumbersome, labour-intensive processes carried out by highly trained personnel making chemical and biochemical information largely inaccessible on a routine basis. The hope is that these laboratory tasks can be miniaturized, integrated and fabricated in silicon. A joint venture between Caliper and Hewlett-Packard has been announced to help them design, develop, manufacture and distribute this LabChip™ technology.

The combination of simple fluidic channels and electro-osmosis are being used to develop integrated biochemical processing systems. Complete designs of interconnected channels are etched into glass, silicon, quartz, or plastic to produce 'hard-wired' experimental protocols that allow the same steps performed at the conventional laboratory bench to be done in volumes as small as a few picolitres. Cell sorters have been produced by micro-fabricating three-dimensional structures within these channels. The structures have 2 to 4 micrometer geometries that can trap particles flowing through these obstacles. Erythrocytes can easily squeeze through these structures whereas the less flexible white cells become trapped. Caliper is also developing microfluidic channels for dispensing molecular separations using electro-osmosis to pump fluids through interconnecting channels. Fluid flow can be controlled using electric fields to create valves at intersections in these channels. Since the geometry of the channels are in the order of 80 μm wide and 10 μm deep, nanolitre quantities can be controlled with the ability to bring together reaction mixtures in picolitre concentrations and in a precisely controllable manner. Molecular separations under these conditions can occur within milliseconds.

Biosite Diagnostics are developing a capillary based lab-on-a-chip technology. The Company's primary product is Triage Panel for Drugs of Abuse, which provides the simultaneous detection in 10 min, of 8 of the most frequently abused drugs and their metabolites in urine including methadone, benzodiazepines, cocaine, amphetamine, opiates and barbiturates. New developments include tests for waterborne bacterial pathogens that can cause severe gastrointestinal infections and their Triage Parasite panel that in twenty minutes detects the presence of *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica/dispar*. Other products include, the Triage Cardiac System that may aid in the diagnosis of Acute Myocardial Infarction and the Triage C. DIFFICILE Panel which is a rapid test designed to identify the common antigen of *Clostridium difficile*, an opportunistic pathogen of the intestine and toxin A, the toxin it produces.

ACLARA BioSciences, formally known as Soane BioSciences, is also applying advances in microfluidics and capillary electrophoresis to genetic analysis, drug screening and optimization, and clinical diagnostics. It is using micro-channels to create miniaturised laboratory-on-a-chip devices where electronic control is used to recreate the processes that occur in analytical laboratories on a smaller scale. The advantages gained by miniaturization are in the reduction of time taken for reactions to occur and the smaller volumes of reagents and sample required. ACLARA is producing low cost plastic biochips by making their technology ideal for mass production. The chip production process involves microfabricating a silicon master, forming a metal mould from this master and

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then embossing the chips into polymer substrates. These chips have been shown to produce high-resolution electrophoretic separation. By announcing a partnership with Perkin-Elmer, ACLARA is in a good position to develop and commercialise micro-fluidic systems for genetic analysis.

Orchid Biocomputer, a spin-off from the Sarnoff Research Centre has developed a micro-fluidic system to revolutionize chemical synthesis and biological analysis for drug discovery and diagnosis. It has developed its biochip in collaboration with Smithkline Beecham creating a credit card sized device that can simultaneously carry out thousands of chemical reactions to identify new drugs. A recent partnership with Dynal has given Orchid access to its magnetic bead technology.

By micro-fabricating arrays of channels, valves and reaction chambers, combinatorial chemistry can be used on a micro-scale to synthesize large numbers of compounds and screen them in parallel. The cost savings derived from the time reduction involved in simultaneous synthesis and high throughput screening will enhance the drug discovery process. Savings are also foreseen in reduced reagent costs, less damaging waste products formed and the reduction in the need for labour and time-intensive handling systems. The miniaturized laboratories presently contain 144 chambers with arrays of 200 μm wide channels through which chemicals can be pumped from reservoirs into reaction chambers using electric fields. These electrohydrodynamic forces can be used both to pump the fluids and to act as valves without the need for moving parts in the micro-structures. The multilayered structures are micro-fabricated in glass, silicon and plastics using laser machining, and chemical etching processes. Future devices will increase the array density to further reduce costs in high-assay throughput.

An alternative approach to miniaturizing DNA analysis techniques that have already been outlined, is being taken by Aura Diagnostics and P&B (Microtech). By exploiting the electrokinetic properties of whole cells, they are developing low cost biochips to identify, detect and separate micro-organisms, cells, viruses and proteins from biological media using their characteristic electrical properties. The success of using these biochips for clinical diagnostics of infectious micro-organisms and disease states depends on the production of automated, low cost disposable devices. New micro-fabrication techniques that allow for the production of multi-layered structures in polymers to machine micro-channels and micro-electrode structures are being exploited. Key advantages in the exploitation of electrokinetic phenomena are the capabilities of sampling handling at the single cell level, and the identification of viability at such a level.

The functioning of these 'biofactory-on-a-chip' devices relies on the movement of bioparticles in non-uniform electric fields (Pethig, 1991). The specificity of the technique arises from differences in the dielectric properties of cells that are a result of differences in their biochemical and biophysical properties. These differences can be used to manipulate the bioparticles, even at a single cell level, without the necessity for any chemical modification processes. These devices do not need to use electrohydrodynamic forces since they exploit the dielectric properties of cells to control movement due to the phenomena of dielectrophoresis, electrorotation and travelling wave dielectrophoresis. Examples of applications of the biochips include, the concentration and viability determination of *Cryptosporidium* using a combined travelling wave dielectrophoresis and electrorotation device (Goater *et al*. 1997), an electrorotation assay for microbial contamination and DNA analysis (Burt *et al*. 1996) and the dielectrophoretic separation and enrichment of CD34+ cell subpopulation from bone marrow and peripheral blood stem cells (Talary *et al*. 1995). To address the requirement for low cost, disposable devices, current work is being directed towards the use of excimer laser ablation to micro-fabricate multilayer devices (Pethig *et al*. 1998).

MICRO-FABRICATION TECHNIQUES

Etching

The most common technique for producing micro-structures is that of a chemical etching process, briefly explained in Fig. 1. Initially the desired material substrate is cleaned and coated with a resist material (Fig. 1B). The resist, which is usually polymer based, is sensitive to some form of radiation exposure e.g. ultra-violet light. When the resist is exposed to the radiation its chemical structure changes through an alteration in the polymerization of the material and becomes chemically resistant (Fig. 1C). In the fabrication process, desired sections of the resist are exposed by projecting the UV light through a patterned mask. This causes the mask pattern to be transferred to the resist. Having exposed the resist, it is then developed whereby exposed regions are attacked by the developer and removed (Fig. 1D). The unexposed regions are resistant to the action of the developer. At this stage the required etch pattern has been fully transferred to the resist and only sections of the substrate that are required to be etched are exposed. The substrate is etched using a chemical caustic to the substrate material which, at the same time, does not affect the resist material. Since only the exposed surface of the substrate is etched the original mask pattern is transferred to the substrate (Fig. 1E). The final stage is the removal of the resist, which is usually carried out by washing in an organic solvent to leave just the micromachined substrate. Chemical etching has

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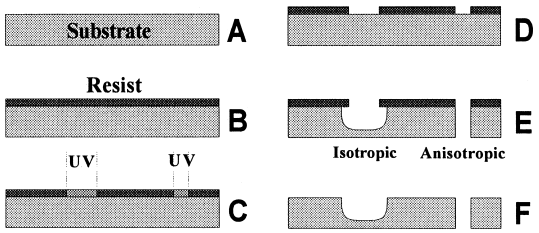

Fig. 1. Chemical etch process showing undercut in isotropic etching and near vertical walls in anisotropic etching of substrate with crystal plain structure.

been employed in numerous applications and the choice of resist materials and etchants is vast.

The substrates that can be etched are also virtually limitless including traditional silicon, glasses, ceramics, plastics etc. The choice of resist and etchant is determined by factors such as the resolution of the desired micromachined feature, the manner of exposing the resist (UV light, electron beams, X-rays) and the depth of the etched feature. In most cases the reaction between the substrate etchant and the substrate produces an isotropic etch. Such an etch removes material in all directions and hence produces a feature which undercuts the initial resist feature (Fig. 1E). Such etching processes can be reasonably accurately managed by controlling reaction conditions such as temperature and duration of the etching process. However, in some cases it is possible to use an etchant which will only etch along certain crystal planes within the substrate material. By orientating the substrate appropriately it is possible to achieve precise etches in the substrate with near vertical walls. This form of etching in only one direction is referred to as anisotropic etching and is particularly popular in etching silicon. Wide ranges of techniques are available to control the etching process. Environmental conditions can be used as a simple control of the etch process by using etch-resistant materials at certain points within the substrate whilst the use of metal bilayers can produce electrode potentials to stop the etch process. Wet etching is a low cost fabrication technique, undertaken with minimal equipment that can readily reach micron resolutions under correct conditions. However, the isotropic nature of many of the etch processes is undesirable when fabricating many laboratory-on-a-chip modules such as precise fluidic channel structures.

A technique gaining popularity, particularly in micro-structure fabrication, is that of dry etching. The most common form of dry etch is the reactive plasma or reactive ion etch where a gas is ionized using a high-frequency voltage to produce a plasma containing a mixture of electrons, ions, neutrons and reactive radicals. The choice of gas determines which materials can be etched. When the substrate is exposed to the plasma, species within the plasma combine with the substrate to produce volatile reaction products that evaporate and hence etch the substrate (Lawes, 1998). The regions of the substrate which undergo the etching process can be predetermined using a resist mask produced in a similar manner to those used for wet etching. The samples to be etched are placed onto a metal plate, which is in turn connected to a high frequency voltage supply. A second electrode above the samples is connected to zero volts. With the etching gas at low pressure, the plasma is created and the reactive species within the plasma are attracted towards the samples by the electric field between the electrodes. As the ions in the plasma hit the substrates, the etching process occurs. Due to the ions being attracted towards the samples by means of the electric field, reactive ion etching tends to be anisotropic in its nature and can allow structures up to 1 mm to be produced. In comparison with wet etching techniques, reactive ion etching is a more complex process to control and has high equipment costs. However, it has lower chemical cost and produces cleaner results with a greater degree of automation available.

Ablation

A relatively new technique that is growing in popularity in laboratory-on-a-chip fabrication is that of excimer laser ablation (Pethig *et al*. 1998). Conventional laser systems tend to use a continuous beam of light to cut or machine by melting or vapourizing materials. Such machining techniques cause damage to surrounding areas of the material and are not conducive to accurate micro-machined systems. Excimer lasers produce very short pulses of ultra-violet light at high energy (typically < 20 ns pulses 300 mJ/pulse). The effect of the pulses on a wide range of materials is to break atomic bonds and so remove a small amount of material with each pulse. Due to the high energy and nature of the ablation, damage does not occur to surrounding material. Laser ablation systems tend to work by focusing the laser beam to a small spot and using the pulsed beam to serially write a desired pattern onto the surface of a substrate. An alternative method is to make use of a large area beam projected through a mask and optical system to transfer the mask pattern to the substrate. Large area mask patterns can be transferred by, either moving the beam step-wise across the mask and repeating the exposure, or by the synchronized scanning of the mask and substrate under a fixed beam.

The ablation action can be accurately controlled by adjusting the energy and the number of pulses of the beam that are applied to the substrate. At low energies, the energy is too low to have any effect on the substrate material. However, at a defined threshold energy, the ablation process occurs. As the energy is increased, the depth to which the material is ablated also increases. For most organic materials

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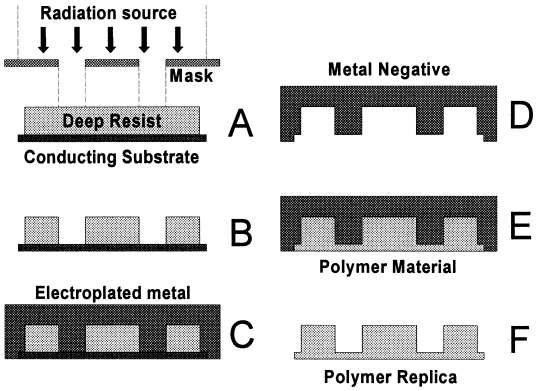

Fig. 2. LIGA process showing the production of a metal 'negative' from which low-cost replicas can be fabricated.

(polymers etc.) this trend is linear allowing a combination of laser energy and number of pulses to accurately determine the depth of machining. By adjusting the optical system used to deliver the laser beam to the substrate the ablation process can machine openings with either vertical walls or under or over-cut walls. When integrated with scanning mask and workpiece stages an excimer laser micro-machining system is capable of complex 3-dimensional profiles and openings in a wide range of materials such as polymers, glasses, ceramics, and thin metal films.

Focused ion beams

Focused ion beams are commonly used in micro-electronic prototyping. They have the ability both to remove and deposit materials. In micro-electronics, they are used to alter prototype integrated circuits by breaking or making electrical connections and trimming components such as capacitors and resistors without the need to repeat production processes. This same ability of removing and depositing materials is of obvious use in laboratory-on-a-chip fabrication. Ions are electrically accelerated and focused using electrostatic fields to produce a fine beam spot of between approximately 10 nm to 0·5 μm in diameter. As the ion beam hits the surface of a material, atoms are sputtered off the material so machining it. By scanning the beam, large areas can be machined with precision. If the ion beam is used in the presence of a precursor gas, materials such as metals or insulators can be deposited onto the substrate allowing complex structures to be created. Like the excimer laser, the fact that the ion beam can use a single beam focused to a spot implies that the machining is carried out in a serial mode, rather like a pen plotter drawing, rather than needing a predetermined mask for projection. Focused ion beam systems are just beginning to find applications in micro-fabrication with distinct advantages over dry etching techniques such as reactive ion etching.

Deposition

Modern micro-engineered devices usually require several layers of different materials to produce the wide range of desired structures. To achieve this multilayer nature, a number of deposition techniques are available for laying down thin layers of metals and insulators.

Metals are usually deposited using either an evaporation or sputtering process. In evaporation, the metal is placed in a crucible close to the substrate to be coated in a vacuum chamber. As the crucible is heated, usually by passing an electrical current through the crucible, the metal melts and evaporates. The metal then condenses on all surfaces within the vacuum chamber, including the substrate. While the simplest method of evaporation is thermal, focused electron beams are also commonly used to prevent the deposition of any impurities which may be present in the crucible. Electrons are accelerated using either electrostatic or magnetic fields and the resulting high energy electron beam is scanned over the metal to be evaporated causing atoms to be ejected from the metal surface. An alternative but slower method to evaporation is sputtering. Again the sputtering process occurs in a vacuum chamber, where a gas, such as argon, is excited to form a plasma using a strong electric field. The ions within the plasma hit a metal target resulting in metal atoms being sputtered from the target. The metal atoms then condense on the substrate to produce a thin metal film.

Insulating materials such as glasses can also be sputtered. However, a more conventional deposition method is Chemical Vapour Deposition (CVD). CVD is the formation of a solid layer from the gas phase via a chemical reaction. In its simplest form, the substrate to be coated is exposed to a precursor gas at an optimum temperature and pressure. This exposure causes a reaction to occur, the product of which is the desired solid film and some gas by-products. A commonly deposited insulator is silicon nitride due to its properties as a barrier to water and sodium ions. Silicon nitride (Si_3N_4) is deposited by reacting dichlorosilane gas ($SiCl_2H_2$) with ammonia gas (NH_3) at a temperature of between 700 °C and 800 °C. The by-products of the reaction are hydrogen gas and hydrogen chloride gas. Similar style reactions are commonly used for insulators such as silicon dioxide and polysilicon.

Polymer layers are usually deposited by spin-coating techniques. These layers tend to be much thicker (0·1–10 μm) than chemically deposited films. In spin-coating, a quantity of the polymer is placed on the substrate in a liquid form, which is then spun at high speed. The rotational action causes the polymer to spread evenly over the surface of the substrate. The polymer is subsequently solidified by either exposure to a polymerizing agent or heating.

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LIGA

All laboratory-on-a-chip devices will have to gain acceptance in the market place to be commercially successful. One of the main considerations in gaining this acceptance is the cost of the devices. Economies of scale only occur when units can be mass-produced. One fabrication technique aimed at addressing this mass production issue is LIGA (an acronym for the German description of the process, Lithographie, Galvanoformung, Abformung). LIGA uses lithography, electro-forming and moulding processes to produce large numbers of micro-structures as described in figure 2. The original LIGA process uses X-rays from a synchrotron source to pattern a thick X-ray sensitive resist on a conductive substrate (Lawes, 1998). This technique is capable of producing high resolution, high aspect ratio structures but at high cost. Adaptations of the original LIGA process have made use of collimated ultra-violet light or excimer lasers to produce the desired pattern in a thick resist. This considerably reduces the cost of producing the original structure at some aspect ratio and resolution loss. Having created a master pattern in the thick resist, the structure undergoes an electro-forming stage where metal is electrochemically deposited over the structure from the conductive base upwards (Fig. 2D). This produces a strong, metal, 'negative' of the original structure, which can be used as a tool for the mass production of replicas. The metal negative can be used in a wide range of production techniques. Commonly used processes include injection moulding to produce low-cost replicas in polymer materials and embossing to produce polymer and ceramic replicas (Fig. 2F).

Assembly

In many laboratory-on-a-chip designs a number of stages within the chip are fabricated separately using a number of manufacturing methods. The final stage of production is a general assembly stage where the complete device is packaged. Two techniques are commonly used for assembly. The first is particularly applicable to silicon and glass fabricated devices and uses a technique known as anodic or electrostatic bonding. The two surfaces to be bound together are placed in contact with each other and heated to a high temperature. A strong electric field is then applied across the joint causing diffusion of ions across the junction resulting in a strong bond between the two surfaces. More conventional assembly techniques make use of adhesives, which are more tolerant of uneven surfaces and provide a compressible cushioning layer to seal devices. This is of particular use when producing laboratory-on-a-chip devices that are likely to be used with fluids. The curing of the adhesives can be carried out using a number of methods such as heating, ultraviolet light, radio frequency heating and general pressure. A major problem still to be adequately solved is the issue of handling such small complex devices. Since there are very few laboratory-on-a-chip devices being manufactured in reasonable quantities, most of the assembly is carried out manually so contributing significantly to the cost of each unit. As market demand for laboratory-on-a-chip devices increases such handling issues will have to be addressed for devices to be economic to use.

COMMON LABORATORY-ON-A-CHIP SUB-UNITS

Over the past decade a number of research groups have developed a wide range of micro-fabricated units for incorporation in fully integrated laboratory-on-a-chip systems. The development of these systems offers advantages in the standardization of methods for laboratory techniques. Here we will discuss several of these units, particularly those which are direct replacements of standard laboratory tools.

Micro-filters

One of the most essential items within a laboratory is a filter. Common filter systems make use of a fine weave of material, which has the ability to trap particles above a certain size. The disadvantage of such systems is recovery of the particles and clogging of the filter, due to the weave-like nature of the filter. As described in earlier sections, micro-fabrication techniques have the ability to precisely machine features in materials with sub-micron resolution. This gives the ability to produce precisely controlled filter modules. Micro-filters can readily be fabricated with not only uniform pore size but also uniform pore distributions over the whole membrane. Using silicon as a base material, bulk etching can produce a membrane of approximately 10 μm thickness. The rigid nature of silicon as the base material also allows the filter to withstand comparatively large pressure differences. The use of reactive ion etching has the ability to produce pores within the filter with sub-micron diameter and near vertical side walls. An added advantage of a micro-engineered filter is the ability to readily apply a cross-flow over the generally smooth surface of the membrane, so reducing the filter blockage and aiding the retrieval of filtered particles such as cells, viruses and macro-molecules. A simple micro-filter is shown in Fig. 3.

Micro-mixers

Efficient mixing of chemical species is also of concern in routine laboratory procedures. The uniformity of the mixing process can determine not only the speed of a reaction but also the accuracy of any result. Inefficient mixing can lead to false or variable results as well as reproducibility and quality control issues.

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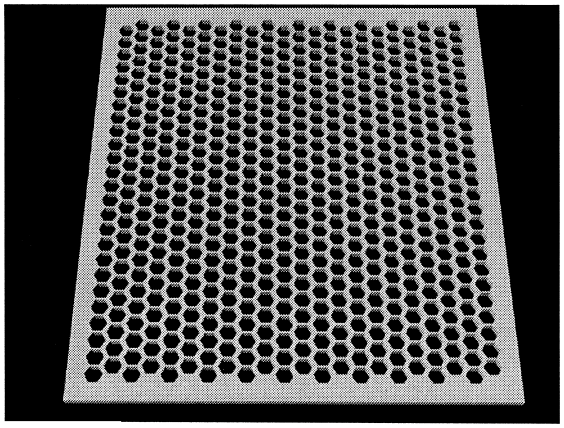

Fig. 3. Micro-filter in 10 μm thick silicon where the smooth surface of the membrane allows retrieval of particles by applying a cross-flow.

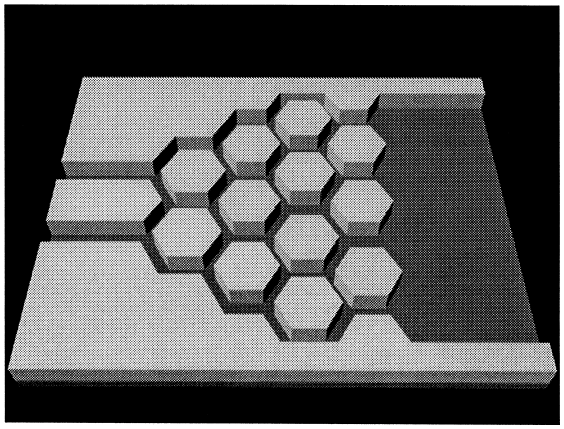

Fig. 4. Micro-mixer for mixing 2 chemicals.

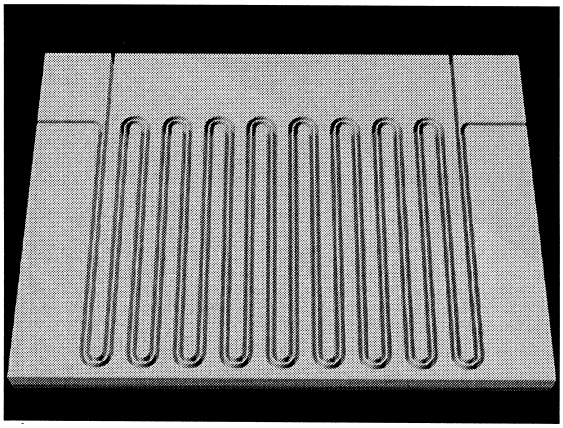

Fig. 5. Heat exchanger showing 2 fluidic channels running in parallel to allow for heat transfer.

In laboratory-on-a-chip type devices mixing is usually achieved using a diffusion controlled process rather than actively stirring a mixture. Fig. 4 illustrates a simple mixer where the two chemical species are fed into the mixer via a group of inlet ports. These ports lead to a mesh of fluidic channels that have the effect of splitting and recombining the streams many times, as the two chemicals pass through the mixer. The splitting and recombining of the sample streams has the effect of coarsely mixing the samples whilst the thin micro-channels promote diffusional mixing of the 2 species. Further mixing action can be achieved by the introduction of structures within the channels that cause turbulence. Such structures could include fine structures on the walls of the channels and twists in the micro-channels.

Heat exchangers, heaters and coolers

The ability of micro-fabrication techniques to produce fine micro-fluidic channel structures opens the way to the construction of a wide range of sample processing modules. Fig. 5 shows a schematic diagram of a simple heat exchanger. Two fluidic channels are fabricated running parallel to each other in an 'S' shaped configuration. One of the channels is used to either transport a sample or store a sample during a reaction. The second channel is connected to a reservoir containing the heat transfer fluid. Due to the large surface area of the fluidic channels in close proximity to each other, heat either passes from the sample to the heat transfer fluid or *vice versa* depending on the relative temperatures of the two fluids. Actively pumping the heat transfer fluid assists in the heat exchanging process.

One of the controlling factors in the efficiency of micro-heat-exchangers is the ability to raise and lower the temperature of the heat transfer fluid. The use of a large reservoir for the heat transfer fluid buffers any rapid changes in temperature. Heating can be achieved by the use of a micro-fabricated heating-coil. Like a domestic electric heater, passing an electric current through a wire causes the wire to heat up. In a micro-system, coils can be accurately formed with defined metal dimensions and composition giving rise to a highly controllable heat source. Due to the inherently small dimensions of laboratory-on-a-chip devices, thermal coupling between the heater and reservoir is very efficient. Cooling can be carried out by the use of heat-sink devices. These structures provide a large surface area for the transfer of heat to the surrounding atmosphere. Heat-sinks are commonly used for dissipating heat in a wide range of electronic systems. A combination of both the small sample volumes in laboratory-on-a-chip devices and the precision of heatsinks allow cooling to ambient temperature with ease.

A method of both heating and cooling can be achieved by incorporating peltier-effect heat pumps within a micro-structure. Peltier devices comprise of a semiconductor material which, when connected to a DC electrical power supply, causes a temperature difference to occur across its surfaces. One surface is exposed to the atmosphere, possibly with a heat-sink, while the other is in close proximity to the fluid

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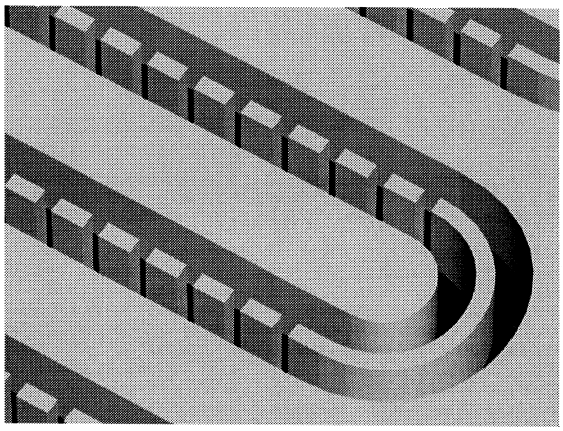

Fig. 6. Micro-extractor where particle extraction is dependent on the hole-size between the 2 fluidic channels.

reservoir. The temperature differential is controlled by the magnitude and direction of the electrical current passing through the device. Reversing the current causes the temperature difference to reverse. By incorporating a peltier-effect heat pump within a micro-system, temperature can be controlled to defined temperatures other than atmospheric temperature and with the incorporation of thermocouple temperature sensors a control of absolute temperature can be achieved. The power transfer of peltier devices can be achieved by using several peltier coolers in parallel as well as by the precise control of the semiconducting nature of the heat pumps themselves. This has proved to be an important development in the fabrication of chip-based PCR tools, as thermal cycling can be rapidly achieved with precisely controlled heating and cooling of the chip.

Micro-extraction modules

Micro-extractor devices allow the transfer of sample subspecies such as macro-molecules etc. from one fluid to another. Fluidic channel systems are fabricated in close proximity in a similar manner to those utilized in heat exchangers. However, in the case of the micro-extraction unit, the 2 fluids are not isolated. By producing holes in the wall separating the 2 fluids, mass transfer can occur between the 2 fluids in a similar manner to dialysis. Control of the transfer process can be achieved by the design of the holes between the two fluids. Larger holes can allow the system to act as a filter where particles such as bacteria may pass from one side of the barrier to another whilst larger cell types are retained in the original sample. The use of smaller holes and the introduction of some driving force, such as osmolarity, can potentially allow microextraction units to be used for processes such as media exchange and macromolecule extraction. An illustration of a simple micro-extraction module is shown in Fig. 6.

Membrane holders

The ability to produce precision membranes has already been discussed. However, micro-fabrication techniques can be applied to conventional membrane systems. Due to the fragile nature of many membrane filters, a mechanical support is required during use. This support under certain conditions can limit the usable area of the membrane. Using micro-machined membrane supports it is possible to achieve the same mechanical support required for the membrane to withstand pressure differentials whilst incorporating fluidic channels into the supporting surface of the membrane holder. Such channels can be used to direct the fluid to the membrane surface and maximize the usable area of the membrane. In addition, due to the fine channel systems achievable using micro-engineering techniques, it is possible to distribute pressure-induced stresses in the membrane more evenly over the entire membrane. This, in turn, can lead to the use of more sensitive membranes (thinner materials etc.) in routine laboratory tests.

Valves and micro-pumping systems

Valves and pumps are an essential item in any automated laboratory equipment. For some time there has been extensive activity in the design and fabrication of micro-machined valves and pumps. The advantages of such devices include the ability to automate laboratory-on-a-chip devices, whilst on a macroscopic scale a micropump is capable of dispensing exact quantities of liquids and gasses. Possible future areas of common use is in healthcare where a patient can receive a continual supply of a drug in nanolitre quantities or the ability to extract nanolitre samples during sensitive investigative procedures.

Both geared pumps and membrane pumps have been successfully fabricated. Geared pumps are usually larger than membrane pumps and use moving parts. This poses a considerable problem in assembly and the device failure time is short, limiting the commercial applications of such pumps. Membrane pumps (illustrated in Fig. 7) are a more commonly used system. These pumps, capable of pumping up to 1 ml/min, work by deflecting membranes to change the pressure in the chamber within the pump. This change in pressure causes the pumped liquid to be either drawn into or pushed out of the chamber. By the sequential alteration of pressure in a number of chambers separated by a series of valves it is possible to pump fluid in a given direction. The valves used to separate the chambers are designed to allow fluid flow in only one direction. The driving action of the membrane is usually either from an electrostatic or piezo-electric force. In an electrostatically driven pump, an electric charge is induced on a membrane, which is then attracted to

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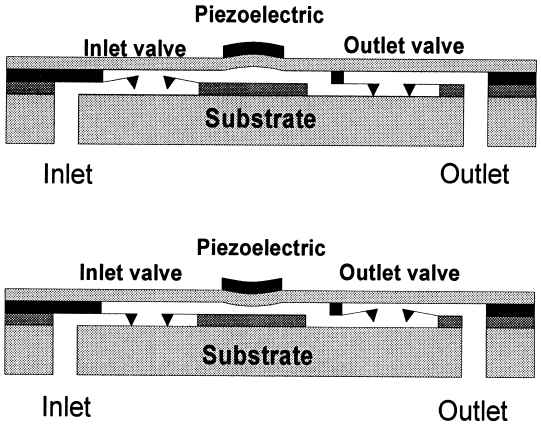

Fig. 7. Piezoelectric micro-pump.

an electrode of opposite polarity. To prevent short circuits in the pump, the membrane is physically restrained from touching the attracting electrode. Piezoelectrically driven pumps use a piezoelectric actuator. When a voltage is applied to a piezoelectric material such as lead-zirconate-titanate (PZT) it expands in a given direction. The extension is usually several micro-metres, however, in a micro-system, this is sufficient to cause a significant change in the volume of a chamber and hence change in pressure. To increase the degree of extension a piezoelectric can produce, bilayer structures are often used. In such a structure, a flexible material is coated on one side with a piezoelectric. As the piezoelectric extends or contracts it causes the bilayer to bend and so amplifying the amount of movement. There are numerous designs of micro-pump and many use pressure differentials as described above as the pumping mechanism. However, some extend this to employ piezoelectrics to actively control valves. In a simple case, a valve can be produced by incorporating a thin, flexible, flap over the entrance orifice of a chamber. If the pressure in the chamber is less than that on the other side of the orifice, fluid is drawn past the flap into the chamber. If the pressure in the chamber is higher than that past the orifice, the flap is pushed over the orifice providing an effective seal. By using piezoelectric bilayers as the flap material in a pump, it is possible to actively open and close the valve without having to change the pressure in the chamber. Such valves can be used to aid the action of micro-pumps or as stand-alone elements to control the flow of fluids around laboratory-on-a-chip devices.

Cell manipulation

A major drawback of many current micro-pump systems is their inability to cope with fluids containing suspended particles such as cells. It is possible to position a pump within a system to minimize the chance of particles entering the pump. However, it is more versatile to use a separate technique to move particles around a chip-based device. Such particle manipulation methods generally use electrokinetic methods to exert a force directly on the particles within the fluid rather than to cause the particles to move as a result of drag in a moving fluid. The more flexible method of electrokinetic particle manipulation is travelling wave dielectrophoresis (TWD). In a TWD system an electrode array is fabricated is such a manner that, when energized with AC electrical signals, a travelling electric field is produced along the electrode array (Talary *et al*. 1996). The electric field induces an electrical dipole in the particle, which in turn causes the particle to experience a force that moves it along the electrode array. The speed and direction of movement is controlled by the electrical properties of the particle and suspending medium along with the magnitude, frequency and phase of the electrical signals used to energize the electrodes. In cells, their electrical properties are determined by factors such as membrane integrity, cell wall structure and surface charge groups. At higher frequencies (greater than 1 MHz) the internal structure of the cell influences the electrical properties of cells. It has been shown that, with optimised electrodes, travelling wave dielectrophoresis can be used for the selective separation of various cell mixtures such as live and dead yeast cells or red and white blood cells. The use of long travelling wave dielectrophoresis electrode arrays can allow sample fractionation procedures to be carried out within a single laboratory-on-a-chip device.

Detectors

Since many laboratory-on-a-chip devices have used silicon substrates, it is obvious that the chip-based diagnostic systems should exploit the electrical flexibility silicon offers to on-chip integrated detection systems. The most popular form of detection system currently used on chip-based devices is optical detection. Making use of the flexibility in micromachining and micro-fabrication techniques, complete optical detection systems can be manufactured on-chip. Lens arrays, mirrors, polarizers, gratings and even interferometers have all been fabricated to allow optical systems to be used for laboratory-on-a-chip detection. Typical detection techniques include absorbance spectroscopy, fluorescence, polarization, refractive index, and surface plasmon resonance. In some cases, light can be generated within the device by the incorporation of light emitting diode (LED) or laser diode structures. However, currently it is more cost-effective to use an external light source and deliver the light to the appropriate location using waveguides and fibre optic systems embedded in the chip. Photo-transistors and charge coupled device (CCD) imagers can also be fabricated on the device to

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provide built in light detection. Advances in this field include the incorporation of signal processing electronics within a final device to minimise interference and increase reliability and usability of the devices.

Also of interest in process detection is the use of electronic or electrochemical detection. Micro-electrodes are commonly used to produce electric fields within laboratory-on-a-chip devices. The use of similar electrodes to measure electrical impedances for the detection of particles or other environmental conditions is a simple extension of macroscopic detection systems. Many electrochemical-based sensors also lend themselves to miniaturization and incorporation into laboratory-on-a-chip devices. Of particular importance is the use of electrical current or voltage measurements to detect binding events such as an antibody binding to an immobilized antigen. This can be of great use in a wide range of chip-based diagnostic devices.

Although there are many advantages to integrating detection systems, their inclusion in laboratory-on-a-chip devices can add significantly to production costs. Because of the differences in processing and material tolerances (aqueous solutions do not mix well with electronics) between micro-structure engineering and micro-electronics, many proposed uses of laboratory-on-a-chip devices do not easily allow integrated detection to be economically included in final devices. To overcome this many laboratory-on-a-chip devices make use of much support hardware for detection and, in many cases, the introduction of samples to the devices.

PCR

It has already been shown that controlled heating and cooling can readily be achieved in laboratory-on-a-chip devices. An obvious biological application of such abilities is apparatus to carry out the polymerase chain reaction (PCR). PCR reactions are routinely used in diagnostic laboratories for DNA analysis, particularly for the identification of pathogenic micro-organisms. Recently laboratory-on-a-chip modules have been described in the literature that perform PCR (Mastrangelo *et al*. 1998). The main feature of the PCR reaction module is a reaction vessel approximately 1 mm^2 with a means of introducing and extracting the PCR products. For the PCR reaction to occur, controlled cycling of the reaction vessel temperature must be possible. As previously described, heating and cooling can be carried out using a number of methods. A very simple method is to use platinum film resistors as a heating source located under the reaction vessel whilst cooling can be carried out using a compressed air flow. Such a PCR module can achieve a 40 °C heating or cooling temperature change in approximately five seconds. Detection of the reaction rate can make use of conventional fluorescence monitoring using either normal laboratory equipment or dedicated LED and photomultiplier based detectors.

CONCLUSIONS

Laboratory-on-a-chip technology is gaining increasing attention due to its potential to revolutionize current laboratory procedures, detection thresholds and analysis times. However, the large-scale use of such systems is several years away. Many of the devices currently in development are relatively simple systems with large amounts of external support hardware. In parallel with commercial development of laboratory-on-a-chip systems, many laboratories are producing a wide range of laboratory-on-a-chip modules which can be thought of as building blocks for future, more complex complete systems. The building blocks are equivalents to current laboratory equipment such as filters, heaters, coolers, mixers and pumps. Due to the enormous costs of developing commercially realistic laboratory-on-a-chip devices, current developments are aimed at high profit markets such as healthcare diagnostics and pharmaceutical development. However, it is hoped that this brief overview of laboratory-on-a-chip technology has demonstrated that the technology can be applied to a vast range of laboratory processes. Whether it is to carry out a complete procedure or optimize a single stage of a protocol, miniaturization technologies can play a part. At present, much of the development work in this subject has been concentrated on device fabrication. By introducing fabrication methods as well as several building blocks in this review, it is hoped that disciplines which will eventually use the technology will recognise its potential and contribute to the direction such technological developments take.

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