Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol. 1 (3), July-Sept, 2011

MICROFLUIDIC CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT

With the fundamentals of microscale flow and species transport well developed, the recent trend in microfluidics has been to work towardsthe development of integrated devices which incorporate multiple fluidic, electronic and mechanical components or chemical processes onto a single chip sized substrate. Along with this has been a major push towards portability and therefore a decreased reliance on external infrastructure (such as detection sensors, heaters or voltage sources)." In this review author(s) provide an in-depth look at the "state-of-the-art" in integrated microfludic devices for a broad range of application areas from on-chip DNA analysis, immunoassays and cytometry to advances in integrated detection technologies for and miniaturized fuel processing devices has been provided.

KEYWORDS: *Integrated microfluidic devices; Lab-on-a-chip; Microfluidic Chromatography.*

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Received:27/08/2011 Accepted: 29/09/2011

INTRODUCTION

Microfluidics deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale.Microfluidics emerged in the beginning of the 1980s and is used in the development of inkjet printheads, DNA chips, lab-on-a-chip technology, micro-propulsion, and micro-thermal technologies. The volume of fluids within these channels is very small, usually several nanoliters, the amount of reagents and analytes used is quite small. This is especially significant for expensive reagents. The fabrications techniques used to construct microfluidic devices, discussed in more depth later, are relatively inexpensive and are very amenable both to highly elaborate, multiplexed devices and also to mass production. In a manner similar to that for microelectronics, microfluidic technologies enable the fabrication of highly integrated devices for performing several different functions on the same substrate chip. One of the long term goals in the field of microfluidics is to create integrated, portable clinical diagnostic devices for home and bedside use, thereby eliminating time consuming laboratory analysis procedures.

THE BASIC PRINCIPLE OF MICROFLUIDICS[1]

The flow of a fluid through a microfluidic channel can be characterized by the Reynolds number, defined as

$$\mathrm{Re} = \frac{LV_{avg}\rho}{\mu} \qquad \text{----(equation 1)}$$

where L is the most relevant length scale, μ is the viscosity, r is the fluid density, and V_{avg} is the average velocity of the flow. For many microchannels, L is equal to 4A/P where A is the

cross sectional area of the channel and P is the wetted perimeter of the channel. Due to the small dimensions of microchannels, the Re is usually much less than 100, often less than 1.0. In this Reynolds number regime, flow is completely laminar and no turbulence occurs. The transition to turbulent flow generally occurs in the range of Reynolds number 2000. Laminar flow provides a means by which molecules can be transported in a relatively predictable manner through microchannels. Note, however, that even at Reynolds numbers below 100, it is possible to have momentum-based phenomena such as as flow separation.

MICROFLUIDIC DEVICES MATERIAL AND POLYMERIC LAMINATE TECHNOLOGY [2]

Silicon has been used extensively to create micro fluidic devices. In it's most basic form, the fabrication technique is as follows. A photo resist (positive or negative) is spun onto and silicon substrate. The photoresist is exposed to UV light through a high-resolution mask with the desired device patterns. After washing off the excess unpolymerized photo resist, the silicon wafer is placed in a wet chemical etching bath that anisotropically etches the silicon in locations not protected by photo resist. The result is a silicon wafer in which micro channels are etched. Often, a glass coverslip is used to fully enclose the channels and holes are drilled in the glass to allow fluidic access.

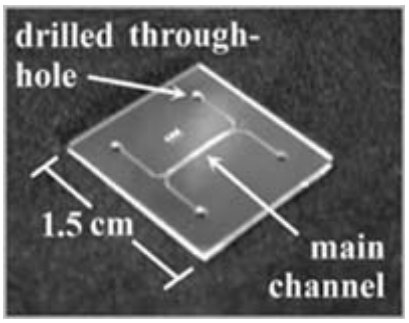

Figure 1: Photogrphic image of Si-Pyrex microdevice

Another popular material for fabrication of microfluidic device is the silicone polymer, polydimethylsiloxane (PDMS). Various plastics are also often used to fabricate microfluidic channels. For, example, hot embossing techniques can be used to imprint patterns into the surface of plastics, or injection molding may be used to create complex structures.

Table 1: Various materials for fabrication of Microfluidic channels and their fabrication techniques

MATERIALS	FABRICATION TECHNIQUES
Silicon	Chemical wet etch
Glass	Chemical etch, Laser cutting
Polymeric Films(Ex. Mylar)	Laminate laser cutting
Silicon Elastomers (PDMS)	Micro molding(“Soft lithography”)
Photo resists, Hydro gels etc	Photo polymerization(Micro fluidic tectonics)
Thermoplastics	Hot embossing, Injection molding

APPLICATIONS OF MICROFLUIDICS[3]

Microfluidic structures include micropneumatic systems, i.e. microsystems for the handling of off-chip fluids (liquid pumps, gas valves, etc.), and microfluidic structures for the on-chip handling of nano- and picolitre volumes. Advances in microfluidics technology are revolutionizing molecular biology procedures for enzymatic analysis (e.g., glucose and lactate assays), DNA analysis (e.g., polymerase chain reaction and high-throughput sequencing), and proteomics. The basic idea of microfluidic biochips is to integrate assay operations such as detection, as well as sample pre-treatment and sample preparation on one chip.

- CONTINIOUS FLOW MICROFLUIDICS [4]: These technologies are based on the manipulation of continuous liquid flow through microfabricated channels. Actuation of liquid flow is implemented either by external pressure sources, external mechanical pumps, integrated mechanical micropumps, or by combinations of capillary forces and electrokinetic mechanisms.
- DROPLET BASED MICROFLUIDICS [5]: The use of electrocapillary forces to move droplets on a digital track is done in this method. The "fluid transistor" pioneered by Cytonix also played a role. By using discrete unit-volume droplets,[6] a microfluidic function can be reduced to a set of repeated basic operations, i.e., moving one unit of fluid over one unit of distance. This "digitization" method facilitates the use of a hierarchical and cell-based approach for microfluidic biochip design. Therefore, digital microfluidics offers a flexible and scalable system architecture as well as high fault-tolerance capability.
- ACOUSTIC DROPLET INJECTION[6][7] :It uses a pulse of ultrasound to move low volumes of fluids (typically nanoliters or picoliters) without any physical contact. This technology focuses acoustic energy into a fluid sample in order to eject droplets as small as a Pico liter. This feature makes the technology suitable for a wide variety of applications including proteomics and cell-based assays. To eject a droplet, a transducer generates and transfers acoustic energy to a source well. When the acoustic energy is focused near the surface of the liquid, a mound of liquid is formed and a droplet is ejected.
- BIOMEMS[8] :(Biological micro electro mechanical systems) Biological matter is manipulated to analyze and measure its activity under any class of scientific study in this tech.
- LAB ON A CHIP[9]: A lab-on-a-chip (LOC) is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size. LOCs deal with the handling of extremely small fluid volumes down to less than pico liters. Lab-on-a-chip devices are a subset of MEMS devices and often indicated by "Micro Total Analysis Systems" (μTAS) as well. Microfluidics is a broader term that describes also mechanical flow control devices like pumps and valves or sensors like flowmeters and viscometers. The basis for most LOC fabrication processes is

photolithography. Initially most processes were in silicon, as these well-developed technologies were directly derived from semiconductor fabrication. Because of demands for e.g. specific optical characteristics, bio- or chemical compatibility, lower production costs and faster prototyping, new processes have been developed such as glass, ceramics and metal etching, deposition and bonding, PDMS processing (e.g., soft lithography), thick-film- and stereo lithography as well as fast replication methods via electroplating, injection molding and embossing.

- APPLICATIONS IN COSMETICS:[10]

- Liposomes and emulsions for delivery of vitamins, peptides, anti-oxidants, etc.
- Cell disruption to release nutrients from plant cells that will be used in cosmetics
- Nanosuspensions including polymers for controlled benefits delivery
- Collagen processing
- Product Quality **-** Control the texture and appearance, improve color depth/range/intensity and improve fragrance encapsulation
- Stability **-** Prolong shelf life, enhance consistent delivery of benefits to specific sites on the body and improve cost-efficiency by running a continuous process using less raw material

- APPLICATIONS IN PHARMACEUTICAL SCIENCES:

- 50% smaller particles than homogenizers
- Improved bioavailability
- Enhanced stability / extended shelf life
- Targeted drug delivery
- Sterile filtration (< 200 nm)
- Less filter material required
- Minimized contamination

DEVICES FOR SEPARATION BASED DETECTION:

GENERAL CAPILLARY ELECTROPHORESIS [11][12]:

A micro fabricated electrophoretic bioprocessor for DNA sequencing, sample desalting, template removal, preconcentration, and CE analysis was prepared.This highly integrated device has been optimized soas to haveas many as 384 separate lanes for capillary array electrophoresis on a single chip. The chip incorporated a number of interesting channel features including low dispersion turns, and detection was done using a 4 color rotary confocal scanner .

Then was presented a PDMS device with integratedhigh voltage electrodes for performing on-chip capillary electrophoresis separations. The platinum electrodes were cast directly into the elastomer prior to curing and the sealed chip was formed by reversibly bonding the PDMS toan etched glass plate. [33]The chip was used to separate DNA fragments and performed a molecular diagnostic analysis of a variety of DNA samples for Duschenne Muscular Dystrophyand cytomegalovirus (CMV) infection. Then a glass CE chip was developed, shown in Fig.2, which fully integrates electrochemical detection and high voltage electrodes and is designed for use with a portable system. The use of microfabrication techniques to integrate permanent electrodes into the chip minimized the number of manual operations required for operation and reduced difficulties associated with variability in electrode placement and geometry.

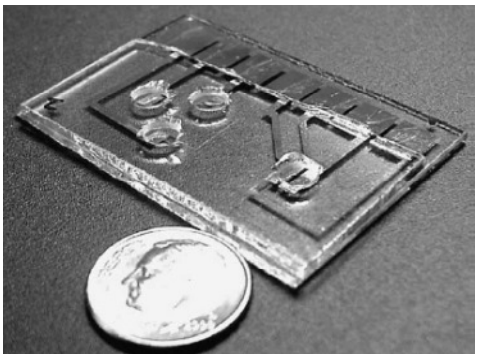

Figure 2: Photograph of a glass CE/EC microchip with integrated electrochemical detection and high voltage electrodes

OTHER SEPARATION AND DETECTION MECHANISMS[13]:

Galloway *et al*. developed a PMMA separation device with an integrated conductivity detector used for monitoring separation (via microcapillary electrochromatography)of double stranded DNA fragments. Prior to bonding, the platinum electrodes were manually inserted into the channel matrix and the device was then sealed with a flat sheet of PMMA. The channel walls were fuctionalized to produce a C18-terminated surface to act as the stationary phase in the separation.. a glass device which integrated two weirswithin a sample channel to form a cavity in which octadecylsilane(ODS) coated silica beads (1.5–4 μm diameter)were trapped for electro chromatography. The design allowed for fast exchange of the microspheres. Then was presented a membrane chromatograph system which consisted of a capillary molded PDMS slab with embedded PVDF (poly(vinylidene fluoride)) membranes adsorbed with BSA. Scientists described an integrated single working electrode PDMS device for the isotachophoretic separation of metal cations. The electrode was integrated into the chip by placing it between the two polymer layers prior to thermal bonding of the two substrates.

A plastic microfluidic system, containing porous poly(vinylidene fluoride) (PVDF) membranes adsorbed with bovine serum albumin (BSA), is demonstrated for high resolution chiral separation of racemic tryptophan and thiopental mixtures. Microfluidic networks on poly(dimethylsiloxane) (PDMS) substrates are fabricated by capillary molding technique. This miniaturized chiral separation system consists of two layers of PVDF membranes which are sandwiched between two PDMS slabs containing microchannels facing the membranes. On-line adsorption of BSA onto the membranes is employed for the preparation of chiral stationary phase and the evaluation of solution conditions in an effort to achieve maximum protein adsorption. Variations in the mobile phase conditions, including solution pH and ammonium sulfate concentration, are studied for their effects on chiral separation. Based on the large surface area to volume ratio of porous membrane media, adsorbed BSA onto the PVDF membranes enables high resolution separation of racemic mixtures with sample consumption of sub-nanogram or less in the integrated microfluidic networks. In addition, the membrane pore diameter in the submicron range eliminates the constraints of diffusional mass-transfer resistance during protein adsorption and chiral chromatographic processes.

MICROFLUIDIC DEVICES FOR IMMUNOASSAY:

Generally a large number of repetitive steps are involved in an immunoassay analysis, resulting in high time and labor costs. As such the advantages in automation and reaction rates offered by micro fluidics are particularly well suited to this application. Currently, the development of integrated devices for immunoassay is significantly less advanced than that for DNA analysis.

Rossier *et al* [14]resented a polymeric microfluidic device with an integrated electrode forenzyme-linked-immunosorbant-assay (ELISA). The integrated electrodes allowed direct in-channel electrochemical detection of the red ox active enzyme substrate. Stokes *et al.* demonstrated a micro fluidics chip with an integrated photo sensor array and associated amplifiers and control

logic for on-chip monitoring of bioassays (specifically *E.coli*). The device used pressure driven flow to introduce detection targets to the reaction chamber where the targets were selectively captured with a series of immobilizedbioreceptors.

A similar integrated circuit DNA hybridization chip was presented in an earlier work by Vo-Dinh.*et al*. . Dodge *et al*. presented an electrokinetically controlled glass microfluidic chip with an integrated reaction chamber for heterogeneous bioassays. Bead based devices have been presented by Choi *et al*., whose device consisted of an integrated biofilter(comprising of a planar electromagnet used to capture magnetic beads that carried the target antigen), an electrochemical immunosensor (an interdigitated array of microelectrodes),and a series of custom designed microval ves integrated onto a glass substrate. Sato *et al*.presented a glass immunoassay microchip that integrated polystyrene beads, precoated with anti-CEA antibody, with microfluidic system using thermal lens microscopy as the detection method. Using this device, reaction times were reduced to as little as 1% of that required for a conventional ELISA.

THE MICROFLUIDIC CHROMATOGRAPH[15][16][17][18]

Embodiments of the invention provide devices, methods and systems for performing microfluidic chromatography. Particular embodiments provide microfluidic chromatography column devices which can perform chemical separation using small sample volumes and low pressure differentials across the column. One embodiment provides a microfluidic chromatography column device comprising a first, second and third capillary tube. A chromatographic packing is disposed in the second tube with a first and second support layer disposed on opposite ends of the second tube. The support layers are disposed in a substantially flat orientation within the tube. An external coupling joins the tubes such that the tubes are fluidically sealed. The device is configured to have a fluidic resistance such that a pressure differential across the column of less than about 10 psi produces a flow rate through the device of at least about 0.5 ml/min for a liquid solution.

Embodiments of the invention provide devices, methods and systems for performing microfluidic chromatography. Particular embodiments provide microfluidic column devices (also referred to as “column devices”) which can perform chemical separation using relatively small sample volumes and low driving pressures (e.g., 10 psi or less). These embodiments can achieve flow rates through the column of 0.5 ml/min or greater to allow for rapid separation of analytes and have relatively small dead volumes to minimize samples volumes and contamination between samples.

An exemplary embodiment provides a column device for microfluidic chromatography comprising first, second and third capillary tubes. A chromatographic packing is disposed in the second tube with a first and second support layer disposed on opposite ends of the second tube. Desirably, the support layers (also referred to as “supports” or “frits”) are disposed in a substantially flat orientation within the column device. An external coupling joins the tubes such that the tubes are fluidically sealed. The dimensions and packing of the column device can be configured such that the joined tubes hold a fluid volume of between about 0.5 to 10 µl, e.g., 0.5 to 5 µl.

The column device is desirably configured to have a fluidic resistance such that a pressure differential across the column (i.e. approximately between the ends of the column) of less than about 10 psi produces a flow rate through the device of at least about 0.5 ml/min for a liquid solution. This flow rate can be achieved when the device is in a vertical or horizontal orientation. The residual volume downstream from the packing is desirably less than 500 nl, and usually less than 100 nl. Residual

volume is the volume of sample solution retained in a portion of device after the solution has been injected into the device. Low residual volumes facilitate the elution of the captured analyte into a very small volume of desorption solution (i.e., the elutent solution), allowing for the preparation of low volume samples containing relatively high concentrations of analyte. Low residual volumes are desirable when the analyte is used in a chemical reactor requiring a minimum volume of analyte, e.g. a reaction to produce a radioactive fluoride compound. Smaller residual volumes also minimize dilution of the analyte, allowing for narrower sampling peaks when the sample is analyzed using any number of detection methods. Desirably, the residual volume of the column device is such that analyte can be eluted off of the packing using less than 20 µl of elutent, and often less than 10 µl of elutent, such as between 5 and 10 µl of elutent. Also, the column can be configured to allow liquid volumes of 10 ml or greater to be rapidly flowed through and separated by the column.

Materials suitable for the capillary tubes includes polymers such as PTFE (polytetrafluoroethylene), silastic or PEEK (polyetheretherketone). The external coupling will typically comprise a heat shrink tubing, such as PTFE. The heat shrink tubing can be placed as an outer tube over an assembly comprising the capillary tubes and supports and then heated to shrink the tubing onto the first, second and third tubes. The heat shrink tubing couples the tubes together via a compressive radial force which also serves to hold the supports in place. Various components of the column device can also be selected to allow operation in high temperature environments such as 100° C. or greater. For example, various thermally resistant polymers can be used, such as polyetherimide, polysulfones, PTFE and related polymers.

The chromatographic packing can comprise any suitable chromatography material, including particles such as alumina or silica particles, porous silica particles and coated particles such as coated silica particles having a chemical coated or covalently bound stationary phase. Suitable stationary phases include ion exchange functional groups (e.g., anion exchange groups such as quaternary amines and cation exchange groups such as carboxylic acids) and various ligands (e.g., C18, C-4 C-8). In certain embodiments, the stationary phase may include immunological (e.g., antibody) groups that specifically bind an analyte, such as a peptide, polypeptide or protein. In a particular embodiment, the packing can include a cationic coating which binds fluoride compounds. In another embodiment, the packing can be an aluminum oxide configured to bind an acid or base as to provide acid/base neutralization of an injected sample. Desirably, the diameter of the packing material particles is greater than the pore size of the support material. The packing material can be configured to separate a first compound from a second compound. The first compound can comprise a small molecule, biomolecule or a reactant. The second compound will typically comprise a solvent in which the first compound is dissolved or suspended. The solutions/solvents that can be used in the column can include aqueous solutions, polar solvents (e.g., DMF), organic solvents (e.g., an acetonitrile solution). In one embodiment, the solution includes a carbonate solution for eluting an adsorbed fluoride compound.

The column device of the invention has a wide variety of uses which will be apparent to the skilled

artisan. The column device is particularly useful for separation and/or purification of small molecules (e.g. molecular weight <500 Daltons), bio-molecules (e.g., hormones, polypeptides, polynucleotides, sugars); inorganic molecules or ions (e.g., flouride, chloride). In one embodiment, the column is used for purification and/or concentration of a radio-isotope (e.g., $^{18}F_9$). The column device can be integrated into microfluidic chips used for chemical synthesis (e.g., production of radiolabeled compounds such as $^{18}[F]_9$-fluoride compounds used in PET scans and other nuclear medicine applications). The column device also can be integrated into microfluidic chips for performing DNA analysis for genetic testing and DNA sequencing; protein analysis for proteomics and gene expression analysis; other chemical analysis for drug and other bimolecular assays, and other uses.

The column device can be configured to be integrated or otherwise coupled to a microfluidic system, such as a microfluidic chip. Typically the column device is coupled to one or more fluidic channels of the microfluidic device. These channels provide inflow and outflow to and from the column device and can be coupled to chemical reaction devices (e.g. a chemical reaction circuit), fluidic delivery devices (e.g., pumps), valves, pressure sources, reaction chambers, reservoirs and sensing devices (e.g., an optical sensor). The column device can also be coupled directly to a pump, valve, or pressure source wherein the tube ends of the column are coupled to these devices using e.g. push fitting, adhesive bonding or other joining method known in the art. The channels can be integral or otherwise built into the chip during chip fabrication or alternatively can be configured to be interchangeable such that one column device can be readily exchanged with another. The shape of the device can be configured to fit on or into a space on the chip such as a well or recess on the chip surface. The column device can be built into the chip or otherwise can be coupled to the chip using micro fabrication techniques described herein or known in the art.

The microfluidic chip can be configured to perform one or more functions which utilize an elutent or other outflow from the column device. For example, the chip can be configured to utilize an eluted solution from the column device in a chemical reaction to produce a desired chemical compound. Also, the column device can be used to perform a chromatographic separation to rapidly produce a concentrated solution of a selected chemical reactant without having to perform an external processing step. This in turn speeds up the processing time on the chip, allowing for high throughput production of the desired chemical products. Accordingly in these and related embodiments, the inflow to the column device can be coupled to a source of dilute solution and the outflow to the chemical reaction chamber. In one embodiment of a microfluidic chip having an integrated column device, the column device can be integrated into the chip so as to rapidly concentrate a radioactive fluorine solution (e.g., from a concentration of 1 ppm to over 100 ppm). This solution is then used in a chemical concentration loop coupled to the column to produce a radio-pharmaceutical such as ^{18}F-flouro-D-glucose.

Embodiments of the column device can also be coupled directly or indirectly to analytical instruments such as, for example, a mass spectrometer, a tandem mass spectrometer or gas chromatograph mass spectrometer. This allows the elutent to be fed into the instrument for further

separation and analysis in either the liquid or a gaseous state. The coupling to these instruments can be though capillary or other tubing or via a spray coupling such an electrostatic spray coupling. In alternative embodiments, the device can be configured to engage an external fluid delivery device such device such as a pipettor, syringe, or external pump.

In an exemplary embodiment of a method for using a microfluidic column device of the invention, where the device is integrated into to a microfluidic chip, a sample volume of solution containing one or more compounds to be separated is injected into the column device via a fluidic channel or other fluid conduction means. The low fluidic resistance of the column allows the solution to flow through the column at rates of 0.5 ml/min or faster using a pressure differential across the column of less than 10 psi. The pressure differential can be generated using a micro-pump or other pressure source. As the sample volume moves through the packing, the compound can interact with the packing in a variety of ways. For example, interaction can occur via hydrophilic, or ionic interactions or chemical adsorption. In the latter case, a desorption solution is injected into the column after the sample volume has flowed through and the compound of interest adsorbed to the stationary phase. This can be achieved using only 5 to 10 µl of solution. Both the sample volume and desorption solutions can be passed rapidly though the column at flow rates of 0.5 ml/min and at pressures of less than 10 psi. For example, a 10 ml volume of solution can pass through the column in 20 minutes or less, a 1 ml volume of solution can pass through in 2 minutes or less and a 5 µl volume can pass through in 6 seconds or less. One or both of the inflow and the outflow from the column can be electronically controlled or otherwise automated, for example, through use of control valves or metering pumps that are coupled to a microprocessor. The inflow or outflow can be synchronized or otherwise temporally linked to another event or process, such as an endpoint in a chemical process or a achievement of a temperature, pressure or flow rate, or rate of change thereof in another portion of the chip. The method can be used to rapidly separate compounds such as proteins, polypeptides, nucleotides, fluorides, halides or other selected compounds.

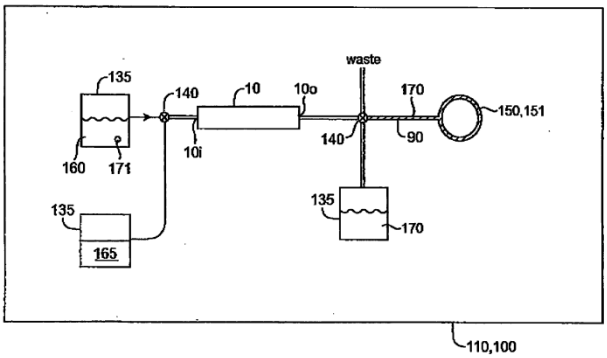

Figure 3: schematic view illustrating an embodiment of the microfluidic column coupled to a chemical reaction device

CONCLUSION:

The objective was to present devices from a broad spectrum of application areas, in order to provide a glimpse into the current state-of-the-art in each of these fields. As we have stated, the majority of micro fluidics research has been concentrated in those areas that have the highest potential for short-term commercial success. In addition to these important applications, we have also examined a few emerging areas that are not commonly covered in reviews of this sort in order to provide a perspective beyond immediate commercial nterests.The next 5 years are likely to be a critical stage in the future development of highly integrated micro fluidic devices. As more and more devices based on microfluidic

technology reach commercialization within this time frame, it is likely the market's response to these early products that will dictate the amount of both private and public funding that will be allocated to the field in the future. Some of

The major developments we foresee within this time period include:

Decreased reliance on external equipment: The majority of the chips described in this review are microscaledevices coupled to a macroscale infrastructure. While this has allowed researchers to benefit from some of theaforementionedadvantages associated with the scaling down of the size, it is highly desirable to decrease the reliance on the external equipment, in order to achieve a higher degreeof portability and hence fully realize the advantages of lab-on-a-chip technology. This requires further development of on-chip raw sample pretreatment capability, miniaturized optical sensors and detectors (e.g., lasers, waveguides, fluorescent microscopes), and low consumption power source.A further increase in the use of rapid prototyping techniques and polymeric construction materials. One of the significant developments in the field during the period covered by this review is the increased use of polymeric materials(as opposed to glass and silicon) and rapid prototyping techniques. These novel techniques and materials have allowed researchers to significantly reduce the time and cost associated with going from idea to chip, and thus are likely tobecome more and more prevalent in the near future. In addition, rapid prototyping micro fabrication techniques require a minimum of expensive, specialized equipment thereby enabling researchers, with a diverse array of backgrounds and potential applications, to enter the field with minima investment.

Increased use of "numerical prototyping" techniques in the design of micro fluidic devices: Simulation allows researchers to rapidly determine how design hanges will affect chip performance, thereby reducing the number of prototyping iterations. Perhaps even more importantly numerical prototyping applied at the conceptual design stage can provide (at worst) order of magnitude estimates of potential chip performance enabling the researcher to take a fruitful path from the beginning. An existing roadblock that limits the use of numerical prototyping techniques is the relatively specialized nature of the low-level numerical tools currently available. These tools typically require sophisticated computational fluid dynamics skills that are not prevalent amongst the chemists and biologists who currently dominate the field. As a result numerical prototyping tends to be an afterthought, rather than an initial step where the greatest gains could be made. To alleviate this, high-level computational design tools, which can be run on a desktop computer, must be developed with the skills of the end users in mind.

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