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Microfluidic Device for Bio Analytical Systems

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Abstract Micro-fluidics is one of the major technologies used in developing micro-total analytical systems (μ-TAS), also known as "lab-on-a-chip". With this technology, the analytical capabilities of room-size laboratories can be put on one small chip. In this paper, we will briefly introduce materials that can be used in micro-fluidic systems and a few modules (mixer, chamber, and sample prep. modules) for lab-on-a-chip to analyze biological samples. This is because a variety of fields have to be combined with micro-fluidic technologies in order to realize lab-on-a-chip.

Keywords: micro-fluidics, bioMEMS, lab-on-a-chip

INTRODUCTION

Though the ink-jet printer and silicon-based gas chromatography had already been developed and used for some time, there was no focus on micro-fluidics until Manz *et al.* proposed the micro-total analytical system in the early 1990s [1]. Now micro-fluidics are used with emerging lab-on-a-chip techniques in a number of fields of biological and chemical analysis, point-of-care tests, and in medical diagnostics. Micro-fluidics is one of the key techniques in the "lab-on-a-chip" field and in miniaturized bio-analytical systems. This device not only incorporates detection but also sample preparation and a digital readout all in one device [2]. Such structures can be manufactured by techniques evolved from those traditionally employed within the microelectronics industry for manufacturing microchips. There are well–known advantages to using a micro total analytical system (μ-TAS) that includes microfluidic systems : 1) Improved thermal and mass transfers due to decreased effective rates of thermal dissipation and effective diffusion, 2) Reduced material consumption requirement due to reduced size, 3) lower external pumping power requirement, 4) Portability, 5) Parallel detection with multiple separation channels on a single chip , 6) Low fabrication costs [3,4].

However, despite the advantages of μ-TAS, the channel dimensions and flow rates typically employed in microfluidic systems lead to a number of performance limitations. One significant limitation is slow reagent mixing, which is due to the fact that the system is restricted to the laminar flow regime (Reynold number <2,000), typically with Re ≈ 1[5,6] (Fig. 1). In addition, the feature sizes are often too small (typically 5~100 μm) to incorporate conventional mixing mechanisms. As a result of this, considerable research is being focused on the design of micro mixers for fast mixing. Another limitation is the required biocompatibility of applied reagents. Therefore, the design of a μ-TAS has to consider in each design step the application of biological molecules such as DNA/RNA, proteins, ionized buffer solutions, and lipids.

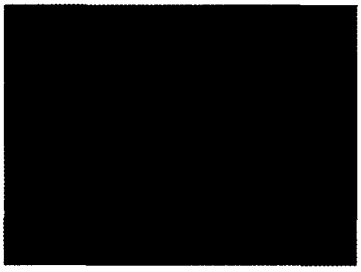

Fig. 1. Micro-channel with two different solutions (1x PBS buffer and rhodamine dye solution, main channel width and depth: 50 μm, material : PDMS, velocity : 10 μL/h).

A variety of fields have to be combined to realize this μ-TAS. Thus, several biological, chemical and engineering techniques are also introduced in this paper to develop μ-TAS for the analysis of DNA, RNA, or protein. This paper describes only a limited number of techniques related to micro-fluidics, and focuses on a few modules of a potential μ-TAS device. However, in both cases, examples are chosen that are important and required for μ-TAS development in the bioanalytical arena.

MATERIALS APPLICABLE TO MICRO-FLUIDICS BIOSYSTEMS

Microfluidic devices like micromixers, micropumps, and microvalves are today's most advanced microdevices and are sold on the market in high volumes. They are used for such things as μ-TAS or nanoliter dosing systems [7]. Silicon is often the material of choice, because well-

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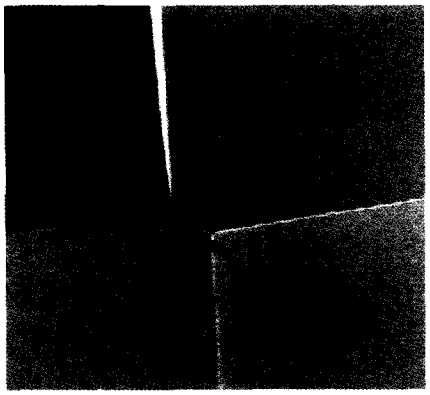

Fig. 2. Silicon substrate etched by UNAXIS770 (depth : 50 μm).

Fig. 3. 3-dimentional SU-8 microchannel.

established standard photolithography technologies including wet and dry etches for microelectronics have been applied in this field. It is possible to make almost any shape and pattern imaginable. Silicon still plays a leading role, not only as the electrical, but also as the mechanical material, as in atomic force microscope tips (AFM). Coupled plasma/reactive ion etchers (RIE) like the Unaxis SLR 770 etcher are used to etch deep patterns in single crystal silicon substrates. The resulting features of RIE are used for MEMS and biological applications. Etch rates of up to 2 μm per minute and aspect ratios of 20:1 can be obtained using photoresist or silicon dioxide as a masking medium (Fig. 2).

Aside from silicon, various types of glass are widely used in microarray and μ-TAS technologies, due to the fact that they are 1) electrically insulating, 2) resistant to many chemicals, 3) microstructurable, 4) transparent at certain wavelengths and 5) coatable with many different metals [8]. Since it is not possible to apply vertical etches to an amorphous material like glass, other channel fabrication techniques than those used with silicon are required. A variety of different methods available to create channels in glass have been developed [9-14]. The easiest way is to use wet etching using Hydrofluoric acid (HF). Often, layers of Cr/Au or photoresists are used as masking agents during a wet etch with HF [9,10]. These layers are fabricated by, and are compatible with, standard lithography techniques using a polysilicon mask deposited by LPCVD (low pressure chemical vapor deposition). There are, however, several problems with the isotropic properties of wet etching in glass, which results in channels with low aspect ratio (<1). Other methods such as laser structuring or sand blasting enable the fabrication of straight walls in glass, but unfortunately they do not meet desired standard batch process requirements and present problems of accuracy.

Photostructurable glass developed and produced at the Ilmenau Technical University [11] has been used to fabricate Capillary electrophoresis chips with modified photolithgraphy technology [12]. This glass was composed of the system Li_2O-Al_2O_3-SiO_2 and modified by micro additions of doping agents (Ag, Ce, Sn and Sb) to achieve UV-sensitivity. The researchers used UV-exposure steps, thermal treatment steps, and etching steps to create the

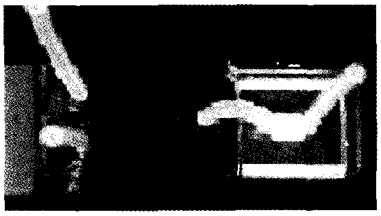

Fig. 4. Micro channel system fabricated with PDMS.

channels. The resolution of this glass is about 5 μm because micro crystallites have a diameter of 1~5 μm of the crystal phase after thermal treatment.

In recent years, the epoxy-based, chemically-amplified resist SU-8 has been used increasingly in μ-TAS development. It has an excellent sensitivity and high aspect ratios [13], and it has a very low optical absorption in the near UV range This makes it very attractive for ultra thick resist applications [14]. This also leads to a relatively good exposure dose uniformly over the entire resist thickness, which gives rise to vertical sidewall profiles and hence good dimensional control over the entire structure height. The aspect ratio of SU-8 structure can be up to 18 with near UV light and a thickness of up to 2.2 mm can be created with a resolution of a few μm (Fig. 3). Since a substantial fraction of MEMS applications do not require submicrometer resolution, the SU-8 photoresist is a very attractive material. It is typically spun on silicon or glass wafers and subsequently patterned with the μ-TAS design.

Finally, the elastomer polydimethylsiloxane (PDMS) has attracted much attention as a material suitable for easy and rapid fabrication of microfluidic devices [15-17] using soft-lithography (Fig. 4). PDMS has a number of advantages. Features on the micrometer scale can be reproduced with high accuracy in PDMS by replica molding technique. It is optically transparent down to 280 nm, cures at low temperatures, and is not toxic. PDMS can also seal reversibly to itself and a range of other materials by making molecular (van der Waals) contact with the surface, or it can seal irreversibly after exposure to an air plasma by formation of covalent bonds. Because it is an elastomer, it will conform to smooth,

Fig. 5. Simulation results: the effects of hydrophobicity of surface on flow in micro channels. (by Fluent/Gambit, left: hydrophobic surface, right: hydrophilic surface)

Fig. 6. Twisted pipe micro mixer [3].

nonplanar surfaces, and it releases from delicate features of a mold without damaging them [15]. Problems arise, however, with high-resolution devices (<20 μm) and accurate alignments due to its sticky nature as well as its inherent hydrophobicity which requires surface treatment for fluidic devices (Fig. 5).

MAIN MICRO FLUIDIC MODULES IN μ-TAS

The development of nucleic acid amplification technologies has made it possible to reconsider the classical ways of performing clinical diagnostics. Potentially, all pathogenic bacteria and viruses are candidates for DNA- or RNA-based detection tests that utilize several nucleic acid amplification technologies. Polymerase chain reaction (PCR) started the revolution of gene-based diagnostics [18]. In recent years, precise PCR themocyclers have been developed for accuracy, and ease of use. In respect to the application of PCR in μ-TAS, it is obvious that the need for thermocycling puts a strain on design and energy consumption. Manz and colleagues have described a unique miniaturized PCR system that could be the answer to some of these constraints [19,20]. Sun *et al.* presented a microchannel chip for continuous-flow PCR using transparent materials This chip consists of an ITO-coated quartz glass etched by standard pholithography and wet-etching techniques and another quartz glass as a lid. An amplification of a 450 bp segment of *Esherichia coli* HB101 was successfully performed by two-stage thermal cyling on a chip device [21]. Lagally *et al.* described a highly integrated glass device for performing multiple PCR and capillary electrophoretic analysis on a chip containing fluidic channels, a capillary electrophesis (CE) channel, and a reactor fabricated by HF-etching techniques. Heaters and thermocouples were then taped to the back of the device to control temperature inside the channel, which demonstrated very rapid thermal cycling (30 s per three stage cycle) [20,22,23].

The detection of DNA harbors the systematic problem that no differentiation can be made between viable and non-viable cells. It is important, however, in the case of food and water safety as well as in clinical diagnostics, to be able to differentiate between viable and nonviable organisms. Some researchers have suggested the use of ribosomal RNA (rRNA) for detection of viable organisms [24-26]. However, it is questionable whether rRNA is a good indicator of viability of an organism because it can persist for long periods in dead cells [27]. In contrast, messenger RNA (mRNA) is turned over rapidly in living bacterial cells, with most mRNA species having a half-life of only a few minutes. This is partly due to the action of degradative enzymes (RNase), which are very stable in various environments [25,28]. Detection of mRNA might therefore be a good indicator of living cells or those only recently dead at the time of sampling. Therefore, reverse transcriptase-PCR (RT-PCR) has been used to measure mRNA for determining viability [29,30]. Obeid *et al.* presented a nucleic acid amplication chip for DNA and RNA to provide very rapid thermal energy transfer and cycling compared to conventional PCR systems [31]. Gel electrophoretic channels were included with the reaction chamber in the system and SYBR Green™ I was used to measure DNA amplified after PCR or RT-PCR. In recent years, a novel amplification reaction has been investigated to fill the gap. Nucleic acid sequence-based amplification (NASBA) can be used to approach mRNA-based technology as an alternative method. It is the commercial development of the transcription-based amplification system (TAS). In 1989, a European patent on NASBA was issued to Cangene Corporation (Mississauga, Ontario, Canada) [32]. Today, Organon Teknika (Boxtel, The Netherlands) holds the rights, title and interest in NASBA. NASBA is a continuous, homogeneous, and isothermal method of nucleic acid amplification designed for RNA. The isothermic nature of the NASBA reaction eliminates the need for such specialized equipment as thermocyclers and makes it thus a perfect candidate for miniaturization.

In μ-TAS devices for the analysis of biological samples, important tasks include the mixing of several compounds such as buffer reagents, enzymes, and nucleic acid probes to create completely homogeneous solutions. As mentioned earlier, the difficulty in mixing fluids on the microscale lies in the small size of the devices. Microfluidic devices that require a mixing operation have typically relied on diffusive mixing by bringing the fluid streams to be mixed together within a single channel. However, flows at the microscale are laminar, thus the efficient mixing obtained in turbulent flows is not practically attainable. The small channel sizes and the lack of turbulence in microfluidic systems has thus led to the development and publication of several static and dynamic mixer designs, often operating under pressure-driven flow [33-35]. In order to mix the liquid well at low Reynolds numbers, the geometry of a channel must be "complicated enough" that chaotic advection can be triggered.

However, in order for fluidic channels to be easily fabricated and integrated into the other microfluidic systems, the geometry should remain "relatively simple". Miyake *et al.* reported a micromixer using a double layers structure for mixing two liquids [36]. Four hundred nozzles were fabricated in the middle of a separating membrane so that one liquid could be injected into the second stationary liquid through these micronozzles. In a different mixing approach, the increase of lateral mass transport with very low lateral diffusion, and therefore several means of increasing the contact area between two liquids, has been investigated [37]. These mixers have multi-stage structures that can repeat the lamination/splitting process. While the mixing capability of such a static mixer without external force to mix solutions may be affected by flow rates and the ratio of the flowing solutions, it is generally more robust and easier to implement than a dynamic mixer in which moving parts will have to be integrated [38,39]. Using a simple analytical model the flow in a "twisted pipe" has been studied [34]. The basic configuration consisted of a sequence of pipe bends with successive bends oriented along different planes causing mixing partly due to chaotic advection. The modeling showed very promising results and the design has been subsequently applied successfully to heat transfer enhancement on the microscale, by fabricating them in PDMS using micromolding and layered manufacturing techniques [40,41]. Kim also showed that a "twisted pipe" has the potential to enhance mixing even at low Reynolds numbers (<100) [3]. This mixing enhancement is possible because of the phenomenon known as chaotic advection [42], in which simple regular velocity fields produce chaotic particle trajectories (Fig. 6). Because the occurrence of chaotic advection typically indicates rapid distortion and elongation of material interfaces, a twisted pipe can increase the area across which diffusion occurs. This leads to rapid mixing by two phenomena typically generated in the channel: segmentation formed by convection and the inter-diffusion of molecules between domains. In contrast to static mixers, the dynamic ones mimic their macro counterparts. Magnetic stirrers, for example, are the conventional dynamic mixer for homogeneously mixing volumes on the order of liters. The tube vibrator mixer becomes the main tool for mixing volumes on the order of millimeters in the biological field [38]. A reliable active micromixer should be tolerant of gas bubbles and the mixing effect should be adjustable by changing the level of an external input force [39]. Approaches using ultrasonic waves have become popular recently [38,43,44]. Ultrasonic force by using lead-zirconate-titanate (PZT), a piezoelectric ceramics, can generate local turbulence near the nozzles under conditions like high frequency operation [38]. However, ultrasonic waves generate substantial heat, which limits their application in bioanalytical systems that require precise temperature control, such as amplification and hybridization reactions of nucleic acid based biosensors.

A different active mixer was introduced by Tsai and colleagues [35]. A microfluidic mixer with a gas bubble filter activated by a thermal bubble actuated nozzle-diffuser micropump was successfully demonstrated. The oscillatory flow induced by the micropump generated a wavy interface between two solutions to increase the contact area of mixing fluids. The effect was demonstrated using a blue food dye and isopropyl alcohol. The alcohol was injected into the blue dye flow using a nozzle driven thermal bubble pump. The motion generated by bubbles expanding and collapsing created a wavy interface between the two liquids. Subsequently, the generated bubbles were removed by bubble filters consisting of narrow and wide channels, thus increasing the surface energy of the bubble when forced through a narrower channel.

Genotype detection using nucleic acid amplification techniques like PCR requires a relatively pure DNA sample in aqueous solution, free of inhibitors during the PCR process. Therefore, sample preparation such as extraction and purification of nucleic acids from biological samples are the critical steps that should be carefully handled in the nucleic acid assays [45]. Also, this series of complex chemical processes are the most difficult and time-consuming part of these experiments. The extraction and purification of nucleic acids from environmental samples, therefore, is a significant analytical challenge due to the co-purification of soluble constituents that inhibit DNA amplification methods such as PCR. Several nucleic acid isolation and purification techniques have been developed to avoid nucleic acid amplification inhibition: centrifugations, precipitations, organic extraction, disposable pipette tips, and others. Most of them, however, are not readily amenable to point-of-care tests (POCT), hand-held automatic devices, or *in situ* environmental monitoring. Recently the developments of capillary gel electrophoresis (CGE) separation and laser-induced fluorescence (LIF) allow DNA separation, purification,and even sequencing in micro channel [46,47]. Matheis' group showed a microfabricated electrophoretic bioprocessor for DNA sequencing, sample desalting, template removal, sample concentration, and detection by CE analysis [47]. Galloway *et al.* [48] demonstrated microcapillary electrochromatography for double stranded DNA fragments viaan integrated conductivity separator/detector made of PMMA. A C_{18}-terminated surface coated surface wall could act as the stationary phase in the separation. Kim *et al.* used silicon-based micro filters to isolate DNA from biological samples [3]. DNA could bind to the exposed SiO_2 surface at a high concentration of chaotropic salt. After DNA binding to SiO_2, ethanol based washing buffer solutions flow through the microchannel of the chip to wash away the remaining sample fluid (Fig. 7).

As briefly mentioned above, temperature control is important for most bioanalytical reactions, especially if a device is supposed to be used in the field under a variety of environmental conditions. Some examples exist already in literature. These typically apply temperature controls to nucleic acid amplification reactions such as PCR for solving problems like slow ramp rate and small sample volume in the microchannels [21,49,50]. Since the temperatures of channels and fluids can affect pressure inside of the device, the velocities of fluids can vary significantly with the temperature gradient when pressure

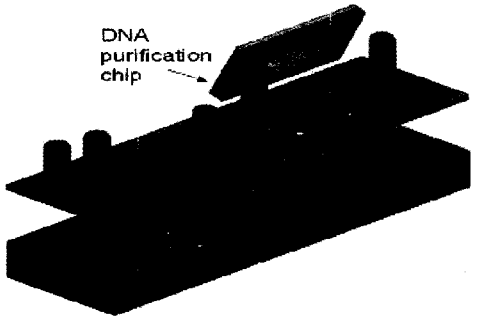

Fig. 7. Micro system- installed DNA purification system.

driven flow is used. Another problem related to effective heating and cooling in microchannels is evaporation, so tight seals for the microchannels are required in order to avoid a loss of even the smallest volumes.

Finally, the connection of the microanalytical system with the macro-world is important, such as packaging, assembling of separate micro-modules, and coupling to external fluidic systems. Two good examples can be found in current literature. A miniature hybrid fluidic circuit board based on plastic bonding was demonstrated by Verlee *et al.* [51]. Epoxy printed circuit board technology or gasketed stacked modules resulted in size reduction and increased integration [52].

COMMERCIAL MICROFLUIDIC PRODUCTS FOR BIOLOGICAL ANALYSIS

Lab on a chip including microfluidics has been developed by several companies for application to bioanalysis because the biotech-related market is rapidly expanding with MEMS technologies.

Caliper Technologies, Inc. have commercialized the first microfluidic Labchip® system, a personal high throughput system, in 1999. A single integrated process replaces multiple manual steps and the need for multiple instruments. This chip requires only nanoliters-scale reagent to show more accurate gel electrophoretic results than with conventional gel methods. Agilent, a partner company of Caliper Technologies, provides the Agilent 2100 Bioanalyzer that can analyse DNA, RNA, and protein with their lab chip®.

Nanogen has developed NanoChip™, which is a 99-site electronically-powered microarray. All samples to be detected are transferred from inlet part to detection part and immobilized by a capture probe. Target DNA then hybridizes the capture probe under the control of an electrical field. Current applications include single nucleotide polymorphism (SNP) and short tandem repeat (STR) analysis.

PharmSeq, Inc. has developed the world's first light-powered microtransponders and nanotransponders with RFID for performing nucleic acid-based assays.

MEMS division in STMicroelectonics is currently working on fluidics for diagnostics. Their first product will be a disposable, standalone, point-of-care, monolithic device, which amplifies DNA in buried silicon channels and detects it on gold electrodes.

Gyros, one of 2000 spin-off companies from Amersham Bioscience, has developed Gyrolab™ : Microfluidics on a CD. This device uses centrifugal force to control the flow in the microchannels. Also, a CD-type array can provide a compact system to manipulate several fluidics at the same time and detect the signal from the microchannels sequentially.

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Other companies such as Cephied, Applied Biosystems, and Aviva Biosciences have also developed bioMEMS-related devices or chips that can be applied to the bioanalytical arena.

SUMMARY

This short review article on micro fluidics in μ-TAS for bioanalytical applications is intended as an overview of basic tools, potential fabrication techniques, and current research trends. Obviously, there are several different ways of realizing a successful μ-TAS, and only a limited number of examples were presented in this paper. Detection mechanisms such as microarrays, which can detect DNA/RNA molecules or proteins, are already commercialized by several companies (Nanogen®, BioMicro systems™). They typically use silicon or glass with standard photolithography techniques and can even be connected to PDMS with molding and soft lithography to create channels and reactors. However, these devices only represent one part of an entire μ-TAS. The integration of the different modules into systems are the main issues to be considered in the near future. Given the impressive application of microfluidics in μ-TAS, there is no doubt that there will be a major role for this technology, which can integrate a room-size lab in one chip, in clinical diagnostics as well as chemical detection.

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