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Microfluidic Chips for Biological and Medical Research

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Abstract—Advantages of devices on a microchip platform are discussed in comparison with traditional systems. Stages and processes of creation of microfluidic chips are considered. The basic technologies of formation micro- and nanostructures on a substrate from various materials and techniques for microchip sealing are introduced. Special attention is given to microfluidic chips for separation and analysis of nucleic acids and proteins, as well as to microchips for PCR. Examples of integrated systems on the basis of microfluidic technique are considered. Data on the commercialization of devices based on microfluidic chips are presented.

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INTRODUCTION

Microfluidics (microhydrodynamics) has developed into an independent branch of science, which studies the behavior of fluids (and gases) at the microscopic level, in 1990s. The advanced modern field of microfluidics is nanofluidics [1] which studies effects in nanosized systems, molecular transport through microchannels, interaction with nanostructures, etc.

The vigorous progress of microfluidics has resulted in the development of instruments capable of reproducibly controlling fluid and gas–fluid flows of nano- and pico-liter fluid volumes in microsized channels [2]. A possibility has thus appeared to implement analytical procedures and develop instruments with technical characteristics different from those of the macro analogs.

The first microfluidic analytical tools on compact planar devices (chips) reproduced electrophoretic separation procedures. Later on microchips realizing other analytical methods, in particular, methods based on the polymerase chain reaction (PCR), were developed. The pioneering R&D in the field of microchip-based PCR systems can be considered to belong to Kricka and co-workers [3–5], whereas the first works on commercialization of microchip technologies for nucleic acid amplification were performed by Northrup and co-workers who demonstrated the possibility of fast PCR on a microchip [6, 7].

In 1994, Ramsey and co-workers [8–11] published their research on electrophoretic and electrochromatographic separation on microfluidic chips. This research group focuses on the development of high-efficiency micro- and nanoengineering systems for clinical diagnostics, high-throughput biochemical analysis, and for solving tasks in pharmacology and bioengineering.

Microfluidic devices offer a number of advantages compared to traditional analytical systems. First of all, this is low reagent and sample consumption, high sensitivity, compact dimensions, and low power consumption.

Note that in foreign literature analytical systems on the basis of microfluidic chips are referred to as Lab-on-a-Chip and micro Total Analysis Systems [12].

In this paper we consider the microfluidic devices for biological analysis – accessible and reliable diagnostic systems not requiring special operating conditions and a highly qualified personnel [13].

In view of the huge body of data on microfluidic systems and their application in biology and medicine, the author does not set himself the aim to give here an exhaustive review, but rather strives to provide a general view of this dynamically progressing field.

Microfluidic Chips

The topology and design of a microchip for biological research are defined by operations to be implemented on the chip. In the simplest case, micro-

fluidic chips represent a construction comprising two plated hermetically sealed with each other: One of the plates contains microchannels, reactors, valves, electrodes, and other functional units, and the other plate fulfills protective functions [14, 15]. An important issue in providing the functionability of a microfluidic device is to organize the motion of fluid flows in microchannels. Generally, the methods and tools for controlling particle and flow motion in microfluidic devices can be classified in terms of the nature of field acting to induce particle and flow motion: force fields (pressure, vacuum, gravitation, centrifugal forces, etc.); electrical constant (electrosmosis, electrophoresis) and alternating fields (dielectrophoresis, electrorotation, etc.); electromagnetic fields (photophoresis, optophoresis, electromagnetophoresis, optical pincers); magnetic fields (magnetophoresis, etc.); and ultrasound fields. Mark et al. proposed a slightly different approach [16]. They recommend to divide devices on the microfluidic platform into 5 groups in terms of the principle of fluid motion: capillary devices and pressure-driven, centrifugal, electrokinetic, and acoustic systems. The way how the fluid flow is controlled, too, defines the topology and design of the microfluidic chip.

Many samples, like blood, have a complex composition, and, therefore, a multistep sample preparation procedure involving separation of particles, cells, or molecules is required. The simplest procedure of particle separation in microfluidic devices is particle size discrimination (filtration), but the particles to be separated should vary in size, and a system of filters is needed for efficient separation [17]. Certain analytical systems are based on CD microfluidic chips [18, 19], where the flows are driven by centrifugal forces. Here the particles to be separated should vary in density. Particles with similar characteristics are separated by other methods [20–34], such as dielectrophoresis [26–28], optophoresis and optical chromatography [29, 30], magnetophoresis [31, 32], acoustic field separateon [33, 34], etc. The most common are electrokinetic methods, but their implementation in microfluidic devices requires choice of control regimes and separation medium, account for possible chemical and electrochemical reactions, etc. [35, 36].

Fabrication of Micro- and Nanosized Structures in Microfluidic Chips

The process of fabrication of an analytical microchip involves the following stages: development of topology and design; fabrication of micro- and nanosized structures on a wafer; formation of film coatings and/or treatment of the surface; control of micro- and nanostructure parameters; fabrication of interfaces; sealing of the resulting structures and components; control and testing of the microchip as a finished device; and packing of the microchip.

The methods of fabrication of microstructures on a wafer are based on the active surface processing of the material (photolithography, laser microprocessing, molding, acid etching, reactive ion etching, etc.) [37–51], which affects the surface properties of the wafer.

The technologies and techniques for microstructure formation have been described in detail in the corresponding reviews and monographs [52–59].

Reducing the cross-section of microfluidic channels to the nano scale should favor more efficient transportation, sorting, and detection of individual biological molecules, like DNA. However, the high fabrication cost and complicated technologies of fabrication, control, and testing of nanostructures hinder the R&D work on nanostructures. At the same time, the use of nanochannels for separation and detection holds much promise in medicine, pharmacology, bioenginnering, and other fields [60, 61]. Thus, microfluidic devices with nanochannels and nanopores offer enhanced possibilities for biological analysis [62]. Single nanochannels and nanopores make it possible to study individual molecules, whereas multiple parallel nanostructures allow concurrently operating with a great number of molecules and gaining a more complete information about the sample. Nanofluidic devices can be used to study conformational, dynamic, and other characteristics of DNA molecules in various media and to gain genetic information along the length of the DNA molecule [63].

The choice of techniques for fabrication of nanostructures in an analytical microchip is defined by the required characteristics and material of the chip wafer. Nanostructures and nanochannels in silicon, quartz, and glass wafers are most commonly fabricated by UV photolithography. There are different combinations of techniques: photolithography and reactive ion etching, photolithography and electron lithography, and others, where optical lithography is used to make a mask for chip fabrication [64]. Optical lithography is a vigorously developing technique, and

the focus is now placed on direct maskless formation of nanostructures. Experimental evidence was obtained to show that exposure to a femtosecond laser pulse produces nonlinear defects in transparent dielectrics, which allows to withdraw ultra-small quantities of the material and to form structures much smaller in dimensions than the laser radiation wavelength. Herbstman and Hunt [65] and White et al. [66] have demonstrated the possibility of direct laser processing of materials to form nanochannels and nanopores in quartz, glass, poly(methyldisiloxane), and diamond film on a silicon wafer. Deep reactive ion etching (DRIE) allows serial production of microelectro-mechanical systems (MEMS) [67] with high aspect ratios (up to 30:1) of channels on silicon plates. This technique was used to fabricate nanostructures for analytical microchips. Some modifications of the DRIE process are known, which allow fabrication of microchannels with higher aspect ratios (40:1) and higher widths (up to 130 nm) [68]. The focused ion beam technique is widely used in microelectronics for maskless production of micro- and nanostructures [69]. This technique is also suitable for direct fabrication of nanochannels for a small quantity of microfluidic chips [70].

Materials for Microfluidic Chips

Microchip devices are most commonly fabricated from silicon, quartz, and glass. The optical characteristics and electrical conductivity of silicon slightly restrict its application in microfluidic chips. Quartz and glass are optically transparent in a wide spectral region, allowing optical detection techniques.

Recently a persistent trend for use of polymer materials, such as poly(dimethylsiloxane), polycarbonate, poly(methyl methacrylate), poly(ethylene terephthalate), polyimide, SU-8 polymer, etc. has become evident [71]. The popularity of polymers is explained by their low net cost and fairly low cost at large-scale production [72]. The techniques for sealing of polymer plates, too, are simpler. However, silicon and glass materials are still on a high demand in the production of microanalytical systems which are required to meet enhanced metrological characteristics.

Microchip devices are frequently fabricated from a Sylgard 184 poly(dimethylsiloxane), an elastomer material with a high optical transparency and a higher biocompatibility compared to silicon. This materials is used for disposable microfluidic chips [73], and it can also be used for sealing of microfluidic chips [74]. Poly(methyl methacrylate) (Perspex, Plexiglas, acrylic glass, etc.) has a good biocompatibility [75]. It is optically transparent and has a lower intrinsic fluorescence than other polymers, specifically polycarbonate. Special thermal bonding techniques have been developed for processing of this material, but their drawback is that they affect dimensions of the structures to be sealed [76]. Search for ways to go around this drawback is being performed. Thus, a technique for solvent-assisted low-temperature sealing of poly(methyl methacrylate) chips has been suggested [77]. Li et al. [78] reported poly(ethylene terephthalate) chips fabricated using plasma surface processing which imparts improvedbonding characteristics to the material. The SU-8 polymer was specially developed for microelectronics and later adapted for MEMS applications. This material is used for fabrication directly in "ordinary" laboratories, without costly engineering equipment, of structures with high spatial resolution and aspect ratio (up to 25) for lab-on-a-chip systems [79].

Sealing of Microfluidic Chips

Silicon plates can be reliably and hermetically sealed by thermal methods (at temperatures above 1000°C) and anodic welding methods (at temperatures varying from 200 to 700°C and voltages varying from 250 to 1500 V).

The processing technologies of quartz and glasses are similar to those for silicon. Glass chips are sealed by physical (high temperatures and pressures, low temperatures, and other factors) and chemical methods. The development of low-temperature sealing technologies [80, 81] was driven by the necessity to integrate plates with electrode, coatings, and thermal sensors. Glass plates with microchannels are generally sealed by thermal welding (sintering) at high temperatures (500–1050 C), anodic welding (70–500°C, 50–1200 V) [82, 83], forming interlayers [84], gluing by polymer compositions, including photocurable polymers [85, 86], optical and deep optical contact methods, etc. In the case of thermal welding and optical contact, high quality requirements are posed on surfaces to be sealed together. To realize these methods, special means and equipments, for example, thermal press, are necessary. Therefore, research effort is directed toward developing simpler and less laborious methods. Zhuang et al. [87] fabricated quartz microfluidic chips by the low-temperature bonding process involving the stage of plate bonding followed by annealing at 200°C for 6 h. Chen et al. [88]

performed low-temperature welding of glass plates after pretreatment with concentrated sulfuric acid under "ordinary" laboratory conditions (the work did not require clean rooms and high-temperature oven).

An alternative approach to sealing microstructures is gluing which offers the advantage of insensitivity to surface admixtures and possibility of joining together plated with diverse functional coatings, including conducting beds. These methods can be developed due to the wide range of UV-curable glues suitable for joining glass with metals and polymers, and, consequently, sealing of microstructures. Such sealing techniques have their special features; in particular, the cover plate should be coated with a thin glue film, whose thickness is only slightly smaller than the depth of channels. For example, Lu et al. [89] described a method for packaging microfluidic chips with a UV-curable glue, involving introduction of the glue and its redistribution in the interstitial space due to capillary forces. Sealing with photocurable polymers can be used in research laboratories for fabrication of small series of analytical microchips [90].

Detection in Microfluidic Chips

The characteristic features of microfluidic analytical methods are small sample volumes and, consequently, fairly low-informative signals. Developers of analytical microchips strive to involve high-sensitivity, high-selectivity, and high-speed techniques. The common detection techniques for biological samples are laser-induced fluorescence [91–93], electrochemistry [94–97], chemiluminescence [98], mass spectrometry [99, 100], Raman spectroscopy [101], interferometry and refractometry [102], etc.

The detection techniques and tools for microfluidic chips have been reviewed in [103–107]. Over the past years new detection techniques, particularly, thermal lens detection, have found expanding application [108–110].

Microfluidic Chips for Separation and Analysis of Nucleic Acids and Proteins

An important line of biological research is studying genetic mutations and polymorphisms responsible for changing gene functions and, apparently, inherited and other diseases. Express DNA analysis is performed by capillary electrophoresis and electrophoresis on a microchip [111–114], mass spectrometry [115–118]; and hybridization analysis on a microchip (biochip) [119]. Electrophoresis on a microchip makes possible high-performance and express analysis of small sample volumes on compact devices which can be integrated into other systems [120–123].

First works on electrophoresis on a chip date back to 1990s [124–126]. In 1994 Woolley and Mathes demonstrated fast (120 s) separation of DNA fragments (271 and 281 bp) on a multichannel microfluidic chip 35 mm in length [127]. Short separation channels in combination with high electric field strengths in microchips make possible faster separation of sample components (up to a few seconds) compared to traditional capillary electrophoresis. Effenhauser et al. [128] made use of electrophoresis on a microchip to separate a mixture of 10–15-bp oligomers on polyacrylamide. McCormick et al. [129] could separate double-stranded DNA fragments within 3 min on a poly(methyl methacrylate)-coated microfluidic chip.

Along with electric field strength, the critical factors for high-performance and high-speed electrophoretic separation of DNA are separation medium and buffer concentration, composition, and pH [130, 131]. For efficient separation of DNA fragments virtually close in electrophoretic mobility in the free state, the polymer matrix of the chip should be optimized with account for the chemical nature and physical characteristics of the polymer and its concentration in the buffer solution [132–136]. Efficient separation on microfluidic chips sometimes requires surface modification of microchannels [137, 138].

Ugaz et al. [139] employed a different technique for DNA separation on hybrid glass–silicon microchips. The separation was performed on a polyacrylamide gel in ultra-short channels (shorter than 1.5 cm) at a low voltage (30 V). The DNA fragments 120–400 bp in length were separated within 85 min. The same research reported a universal microelectrophoretic platform with a great number of electrodes, heaters, and temperature sensors, which allows use of various gel structures to separate single- and double-stranded DNA fragments in a 1-cm-long channel [140].

The Human Genome Project has stimulated development of electrophoretic sequencing technologies. Even though the human genome is considered to be decoded, sequencing technologies are constantly progressing, and new commercial instruments and analytical systems are being developed in the microcale format. Woolley and Mathes [127], using four-color detection, performed DNA sequencing within 540 s in a 3-cm-long microchannel. Liu et al. [141], having

optimized the separation matrix and separation conditions, could perform DNA sequencing with 500 bp read length within 20 min. In [142], DNA sequencing (450 bp read leangth) was performed on a 16-channel microchip within 15 min. These works led to the development of multichannel microfluidic chips. Thus, Paegel et al. [143] made 96-channel microchips with a 16-cm-long separation channel, capable of reading up to 500 bp within 25 min.

In the post-genomic era, the greatest researchers' interest is attached to proteomics. Human proteome mapping, like genome mapping, will serve as a source of useful information for biologists who study the effects of the environment and medicines on human body. High-performance devices fabricated by the microchip technologies hold promise for molecular cell research [144]. Protein research is important for clinical diagnostics, since it provides information on systemic diseases. Proteins are more difficult to analyze than DNA. Moreover, proteins are impossible to amplify, and, therefore, other concentration techniques are required. Protein analysis is a complicated issue, since proteins are sensitive to various physical and chemical factors. The task of protein separation and analysis can be solved to success by means of microfluidic chips.

First successful attempts to adapt microfluidic technique to the analysis of proteins by capillary zone electrophoresis were performed in [145–147]. Later gel electrophoresis, isoelectric focusing [148–152], micellar electrokinetic chromatography, microchromatography [153], electrochromatography [154, 155, and other techniques [156–158] were adapted. The sensitivity of protein detection could be enhanced by the use of nanomaterials [159]. Impressive results in protein analysis were obtained due to integration of microfluidics with mass spectrometry [160].

Electrophoresis on a chip is also used in inorganic and organic analysis [161, 162], immune analysis [163, 164], etc. It is important to mention that one of the promising fields of microfluidic technologies is development of systems for synthesis of biopolymers, including oligonucleotides [165]. Such systems can reduce the cost of genetic research and the synthesis time.

Microfluidic systems are being improved, attention is being paid to development of cheap and accessible analytical devices and their constituent parts, such as microfluidic paper-based electrochemical sensing devices [166, 167].

Microfluidic Devices for Polymerase Chain Reaction

Polymerase chain reaction (PCR) is one of the leading methods of DNA/RNA analysis both in scientific research on gene cloning and DNA sequencing and in applied research, specifically, genetic analysis, diagnosis of dangerous infections and other diseases, identification of genetically modified products, forensic analysis, etc. [168–170].

Up-to-date technologies allow fabrication of microchips with a PCR chamber having a high surface area-to-volume ratio for fast and efficient heat exchange. Microfluidic devices for PCR can be classified in terms of the principle and mechanism of heating of the reaction mixture (direct heating of the chamber, continuous flow heating, convection heating, electromagnetic radiation heating) and the way of transport of the reaction mixture (stationary and continuous-flow systems) [171, 172]. The systems with stationary reaction chambers, i.e. chambers where the temperature changes in every amplification cycle, include single-chamber [173, 174] and multichamber chips [175–181]. Probably, the key problems of functioning of these systems consist in the difficulty in obtaining uniform controlled temperature fields, preventing cross contamination of samples from neighboring chambers, and modifying the surface of the reaction chamber for a more efficient reaction.

Researchers from the Institute of Bioengineering and Nanotechnology (Singapore) proposed a radically different approach to the creation of stationary chambers for small samples. This approach consists in the use of a specially structured hydrophobic–oleophobic surface, where a droplet of a PCR mixture is encapsulated in a larger droplet to form a virtual reaction chamber [182, 183].

In continuous-flow microfluidic chips for PCR, amplification takes place as the reaction mixture moves along the microchannel, passing corresponding temperature zones. One of such PCR chips was suggested by Nakano et al. in 1994 [184]. Later continuous-flow microchips were improved [185, 186]. Unlike what takes place in PCR microchips with stationary chambers, in continuous-flow microfluidic chips the heat inertia is a minimum, since the key factor here is the thermal mass of the PCR mixture. The frequency of temperature cycling depends on the flow rate and stability of the reaction mixture and the thermal equilibration time. Commercial plastic

microfluidic chips for continuous-flow PCR applications are presently produced by Microfluidic ChipShop (Germany).

Continuous-flow reactors have a number of drawbacks: the formation of air bubbles in microchannels adversely affects reaction progress; pressure-controlled flow has a parabolic profile, which entails sample diffusion; the flow rate of the reaction mixture in different temperature zones is difficult to control. Various practices have been developed to overcome these drawbacks [187], and even certain alternative approaches have been proposed. For example, Nakayama et al. [188] proposed to prevent air bubble formation by introducing into channels viscous oil droplets before loading the PCR mixture. Singapore researchers [189, 190] studied the mechanisms of bubble formation in microreactors of varied geometry, with siloxane and glass feed channels, and proposed ways to suppress this effect.

Concurrent amplification of a large number of samples is hardly accomplishable in continuous-flow PCR microchips with helical- or serpentine-shaped channels. Some researchers, for example, Wang et al. [191], proposed a microchip with a linear channel, where the mixture flows in an oscillating manner through three temperature zones. Such construction makes it possible to create a microchip for simultaneous amplification of a large number of samples.

Since PCR microchips have a high ratio of surface area to volume of reaction chambers and channels, reactions between surface and reagents are possible, which affects the efficiency of the reaction. For successful PCR-on-a-chip the inner surface of the reaction chamber or microchannel are modified. The modification techniques can be classified into two groups: static [179, 192–196] and dynamic [179, 188, 197, 198].

There is controversy among researchers about the minimum volume of chamber for efficient PCR. Below we briefly describe microchips with PCR chambers of different volumes. Marcus et al. [199] developed a microfluidic chip for 72 parallel PCR with reverse transcriptase, injection volume 450 pl. Morrison et al. [179] described a 33-nl device for 3072 parallel reactions. Matsubara et al. [175] proposed an integrated silicon PCR device with microchambers, injection volume 40 nl. Neuzil et al. [183] reported a PCR system with virtual reaction chambers for amplification of a 100-nl sample encapsulated in a mineral oil (1 μl). Guttenberg et al. [200] performed amplification of a 200-nl sample in a 5-μl droplet of mineral oil. Landers's group [201] developed a hybrid PCR device with an electrophoretic separation channel and pressure sample injection via an elastomer valve, amplification was performed in 280-nl reaction chambers. Beer et al. [202] described a system, where amplification was performed in a 10-pl oil-encapsulated droplet.

Even though at present there is a clear tendency for miniaturization of amplification chambers, large-volume PCR-on-a-chip systems (> 3 μl) are still being developed and applied. For example, Lee and coworkers [203, 204] have developed PCR microchips with reaction volumes larger than 10 μl. Large-volume PCR reactors offer advantages over small-volume ones in the case of samples with low concentrations of target molecules (for example, in diagnostics) due to low sample losses. However, small-volume PCR analysis is not only less costly, but also less time-consuming.

One of the problems of the PCR-on-a-chip analysis is sample contamination with specific and nonspecific DNA molecules from the environment, which can lead to false-positive and false-negative results. A radical solution of the problem of contamination in repeated amplifications in the same microchip is provided by the use of disposable, generally polymeric, chips. Approaches to preventing contamination have also been developed for reusable microchips. For example, microchips are recommended to be washed after amplification with organic solvents, concentrated inorganic acids, or strong detergent solutions. Cross contamination on PCR microchips with stationary reaction chambers can be prevented by modifying microchip surfaces to endow them with different properties, for example, the inner surface of the reaction chamber can be made hydrophilic and the outer surface, hydrophobic [175]. This approach facilitates loading and isolated retention of the reaction solution in individual chambers. Another protection technique involves sealing the chamber with the sample with a special film, like a polyolefin sealing tape from Sarstedt.

Quite promising are digital microfluidics techniques which generate droplets/bubbles in the PCR mixture and transport them through thermal zones [204, 205].

Integrated Microchip Systems

A natural tendency in the development of microchip systems is the integration of different modules and devices on a microchip [206]. Such integration has its advantages and disadvantages. The advantages include the possibility to isolate the sample from the environment and thus to eliminate mistakes associated with contamination, human factor, and others; reduce analysis time; and provide total control and automation of all stages and operations.

In a general case, biosample analysis involves several stages: separation and sorting of cells, lysis, extraction, purification, and amplification of nucleic acids, and detection of the resulting product. Implementation of these stages requires fully integrated systems, or, at least, systems integrating several functional modules on a microchip. Methods for separation and manipulation of live cells are quite diverse and are successfully developed at present [20, 26, 34, 59]. Special attention is paid to isolation and purification of genetic material, since this stage is critical for successful analysis [207].

Developers of integrated systems not infrequently restrict themselves by embedding separate sample preparation units or devices in a microchip. As a rule, sample volumes are very small, and, therefore, the analyte concentration have to be increased in any way. One of such approaches is the DNA amplification reaction [208]. The integral elements of many microchips are reaction chambers with heating–cooling units for amplification (see, for example, [209]). Polymerase chain reaction requires fairly pure DNA targets, whereas real samples may contain various admixtures. Therefore, microchannels are provided with filters and DNA extraction and concentration units [210–212]. A fully integrated microchip system was suggested in [213]. This system fulfills injection of a sample and its mixing with reagents, DNA amplification, electrophoretic separation of products, and detection. Kaigala et al. [214] developed a partially automated microfluidic chip including a reactor (PCR chamber), separation channel, valve system, micropump, and heater; detection was performed using an external contact device.

The up-to-date engineering level allows fabrication of fully automated systems in the microchip format. However, full integration is a task which requires a complex of technical, engineering, and methodical problems to be solved. Therefore, the development of an integrated analytical system on the microchip platform should be driven by a strong motivation, as such work necessitates considerable investments of money and labor. At present only a few fully integrated microanalytical systems have been developed: for pathogen analysis [215, 216], for medical expert examination and identification of DNA [217, 218], and for space research [219, 220]. In most cases, full integration is inexpedient, because such devices are quite costly. Therefore, at present more widely distributed are comparatively low-cost microfluidic chips with specialized detection systems.

Commercial Microfluidic Devices and Instruments for Biosample Analysis

First publications on microfluidic chips date back long ago, and impressive results have already been reported, but microfluidic devices are not yet commercialized on a large scale. Surely, it should be borne in mind that microfluidics is still a new field, and there are a lot of alternative analytical tools on the market. At present certain companies perform marketing research of and invest money into the projects focusing on the development of lab-on-a-chip systems for biomedical research, medical diagnostics, space research, military technologies, environmental monitoring, etc.

Yole Développement (http://www.yole.fr/) which specializes on the development of databases and prognosis of trends in the development of up-to-date technologies (MEMS, nanotechnologies, microfluidics, etc.) launched on the market the Worldwide Microfluidics Database. According to this database, 269 institutions in 31 country, 118 university groups, and 35 contract research companies are presently involved in R&D in microfluidics. The list includes 3M Asia Pacific Pte and Adhesives Research (USA); Affymetrix (USA); Bio-Rad (USA); Caliper Life Sciences (USA); Corning (USA); Dai Nippon Printing (Japan); Epigem (UK); Fluidigm (USA); GE Global Research (USA–India–China–Germany); IBM Research (Switzerland); ITRI – Industrial Technology Research Institute (Taiwan); Luminex (USA); Micralyne (Canada); Microgen (USA); MicroCHIPS (USA); Pamgene (Netherlands); Roche (Switzerland); Sharp (Japan); Tecan (Switzerland); Texas Instruments (USA), etc. The areas of interests of these companies are quite broad: from the application of microfluidic devices in microelectronic technique and office appliances (printers, plotters, etc.) to advanced medical and space research.

Companies producing commercial instruments for biosample analysis by the microfluidic technology

Company (firm)	Country	Foundation year	Commercial product	Internet address
Aclara Biosciences	USA	1995	eTags Assay System	www.aclara.com
AVIVA Biosciences Corporation	USA	1999	SealChip	www.avivabio.com
Caliper Life Sciences	USA	1995	LabChp, LabChip 90	www.caliperls.com
Cepheid	USA	1996	GeneXpert, SmartCycler	www.cepheid.com
Fluidigm Corporation	USA	1999	BioMark Dynamic Array, BioMark Digital Array	www.fluidigm.com
Gyros	USA	2000	Gyrolab Bioaffy CD	www.gyros.com
Handylab	USA	1999	Integrated cartridges	www.handylab.com
Microfluidic Systems	USA	2001	Microfluidic cartridges and casettes	www.microfluidicsystems.com
Micronics	USA	1996	PanNAT, microFlow System, Active Lab Cards, Access cards, etc.	www.micronics.net
Nanogen	USA	1991	NanoChip	www.nanogen.com
CFD Research Corporation	USA	1991	Microfluidic cartridges, microchips, Pharos software	www.cfdrc.com
Microfluidic ChipShop	Germany	2002	Microfluidic chips, microscopy-slide platform, flow Chip-PCR — *ChipGenie* Support Kits	www.microfluidic-chipshop.com
Fluigent	France	2006	FASTAB technology, EMMA technology, microfluidic control systems, polymers, software	www.fluigent.com
MicroLIQUID	Spain	2006	Microfluidic chips, microfluidic chip holders, prototyping microfluidic systems and connectors	www.microliquid.com
Micronit Microfluidics	Netherlands	1999	Fluidic connect, microfluidic chips, microfluidic tool kit for on-chip capillary electrophoresis	www.micronit.com
Microchip Biotechnologies (now IntegenX)	USA	2003	Microbead capture. Microscale on a chip valves (MOV). Appolo System	www.microchipbiotech.com (http://integenx.com)

Less developed is the field of patenting microfluidic instruments and devices for biological and medical applications. Here the greatest focus is on the development of individual components for sample preparation, enhancement of the efficiency of nucleic acid amplification, development of no-chip separation and detection systems, and miniaturization. The table lists the companies owning the greatest share of intellectual property in the field of microfluidic technologies (the data are taken from [221, 222]).

A few more companies developing microfluidic devices for biosample analysis can be mentioned.

The indisputable leader and pioneer in the development of microfluidic technologies is Agilent Technologies which produces microfluidic chips for DNA, RNA, protein, and cell analysis. The Agilent Technologies and Caliper Technologies in 1999 could develop a commercial system for automated analysis of nucleic acids Agilent 2100 Bioanalyzer by the microchip technology (Caliper LabChip Technology). Bio-Rad (http://www.bio-rad.com) together with Caliper Technologies created a compact bioanalytical system Experion Automated Electrophoresis System on the microchip platform, which allows 10–12 samples to be analyzed within 40 min. In January

2002, Caliper Technologies presented at the exhibition LabAutomation 2002 (Palm Springs, California) a Caliper AMS-90 SE system for DNA fragment analysis, which addressed the needs of laboratories dealing with high-throughput DNA fragment analysis (twin library quality assessment).

The AMS-90SE system allows automated DNA analysis on a microchip with electrophoretic separation and laser-induced fluorescence detection; the system contains internal calibration standards.

In 2002 Shimadzu (www.shimadzu.com) launched a MCE 2010 quartz glass chip for DNA sequencing. The system is equipped with two detectors: spectrophotometric UV detector and laser-induced fluorescence detector. Since 2007 this company has been producing a MCE-202 MultiNA fully automated system for electrophoretic analysis on a microfluidic chip.

Cepheid is one of the first companies which initiated commercialization of integrated systems for genetic analysis, with an iCore rapid thermal cycling module technology with microoptics and electronics. At present the company produces two types of analyzers: SmartCycler and GeneXpert, which are used to success in research laboratories, hospitals, and other institutions for express screening of infectious diseases, cancer diagnosis, and detection of pathogens. Microchip Biotechnologies (now IntegenX) specializes on microfluidic systems integrating modules for sample preparation and automated DNA sequencing. Fluidigm (http://www.flui-digm.com) implements the integrated fluidic circuit (IFC) technology for fabrication of instruments on the microchip platform with a system of channels and low-control valves (NanoFlex valve). These instruments are designed for PCR, protein crystallization, gene expression analysis, etc.

Micronics mastered large-scale production of microfluidic devices for laboratory research, medical diagnostics inclusive. Micronit Microfluidics produces and distributes microfluidic chips all over the world. ThinXXS Microtechnology (http://www.thinxxs.com/), which specializes on the development and fabrication of microfluidic devices from plastics [poly(methylmethacrylate), polycarbonate, polypropylene, etc.] for pharmaceutical, medical, and chemical industries. The company suggests services on the development and fabrication of microfluidic chips by means of precision pressure molding according to customer's topology (or design). Microfluidic ChipShop suggests a wide range of microfluidic chips and diverse auxiliary devices, for example, microchips for continuous-flow PCR and auxiliary devices, including thermal cyclers.

CONCLUSIONS

Microfluidic instrumentation has substantially progressed from fairly simple devices and instruments for electrophoretic separation to systems for single-molecule analysis, devices for manipulation of individual biological objects (cells, bacteria, and viruses), and integrated microanalytical systems. The following trends can be recognized in up-to-date chip-based microfluidic devices: miniaturization; integration of functional modules on a single wafer; increased number of measurement channels and reaction chambers; application of nanosized systems; and total automation and control of all process stages on a chip.

It was impossible to fit in the framework of a brief review all constructions, techniques, and application fields of microfluidic systems for biological and medical research. Microchip systems for research on cells, bacteria, and viruses remained uncovered.

Microfluidic chemical systems continue to progress. A new field has recently appeared, which combines the microfluidic technology and high-resolution microscopy (atomic force microscopy, near-field optical microscopy, confocal scanning microscopy). Microfluidic technologies make it possible to fix the object to be measured, such as a cell, in a chosen place of the microchip [223–225] and to create fluid flow around the cell, while high-resolution microscopy provides information on the behavior of the cell and its response on changing environment, its interaction with other cells, etc. [226, 227].

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