

Microfabrication of biomedical lab-on-chip devices. A review

Athanasios T. Giannitsis

Department of Electronics, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia; thanasis@elin.ttu.ee

Received 1 February 2011, in revised form 8 April 2011

Abstract. Lab-on-chip systems are a class of miniaturized analytical devices that integrate fluidics, electronics and various sensorics. They are capable of analysing biochemical liquid samples, like solutions of metabolites, macromolecules, proteins, nucleic acids, or cells and viruses. Supplementary to their measuring capabilities, the lab-on-chip devices facilitate fluidic transportation, sorting, mixing, or separation of liquid samples. A type of lab-on-chip devices, named biochip, is devoted specifically to genomic, proteomic and pharmaceutical tests. The significance of such miniaturized devices lies in their potentiality of automating laboratory procedures, which highly reduces the time of biomedical tests and laboratory work. This review summarizes numerous fabrication methods and procedures for producing lab-on-chip devices and also envisages future evolution.

Key words: lab-on-chip devices, biosensors, biochips, microfluidics, microfabrication.

1. INTRODUCTION

Lab-on-chip technology focuses on the development of hybrid devices, which integrate fluidic and electronic components onto the same chip [^{1–5}]. They are devoted primarily to liquid sample testing and handling. Such devices reduce laboratory processes in a manner competitive to bench-top instruments. Lab-on-chip technology emphasizes integration, chip programmability, increased sensitivity, minimal reagent consumption, sterilization and efficient sample detection and separation. Lab-on-chip functionality includes sample handling, mixing, filtering, analysis and monitoring. A typical lab-on-chip device contains microchannels, which allow liquid samples to flow inside the chip, but also integrates measuring, sensing and actuating components such as microvalves, microfluidic mixers, microelectrodes, thermal elements, or optical apparatuses.

Some commercial lab-on-chip devices incorporate electronics that operate their apparatuses (Fig. 1). The lab-on-chip devices are considered analogous to the systems-on-chip in electronics.

Biochemists, synthetic chemists and medical doctors currently evaluate the potentiality of lab-on-chip devices in the context of biochemical synthesis, analysis and screening. Specific areas of applications have emerged, from the detection of infectious diseases and diagnostics to the assessment of food quality. Several on-chip clinical assessments include cell analysis, cytometry, blood analysis, nucleic acids amplification, genetic mapping, enzymatic assays, peptide analysis, protein separation, toxicity analysis and bioassays. Lab-on-chip devices find growing applications in drug discovery. In pharmacy, the lab-on-chip devices gradually become valuable in the area of drug research with the emphasis on cell targeting, clinical trials, drug synthesis, pharmaceutical formulations and product management process. The lab-on-chip devices are found promising in the analysis of drugs and determination of optimal dosages. This is especially useful for testing the synergistic effect of combined drugs. Microfluidic networks-on-chip offer unique opportunities to imitate natural veins for testing nanoparticles as drug carriers for targeting cells or, moreover, they offer opportunities for examining in vitro the metabolisms of biological cultures. Technical limitations such as size reduction, sample input rates, power consumption, but also chip reliability and biocompatibility shall all be accounted in the design of lab-on-chip devices.

Lab-on-chip devices have become very attractive nowadays as they force the development of personalized devices for point-of-care treatments [6]. The lab-on-chip technology enables the development of the next generation of portable and implantable bioelectronic devices. Due to their biosensing capability and embedding concept, the lab-on-chip systems are attractive platforms for developing implantable bio-inspired sensors.

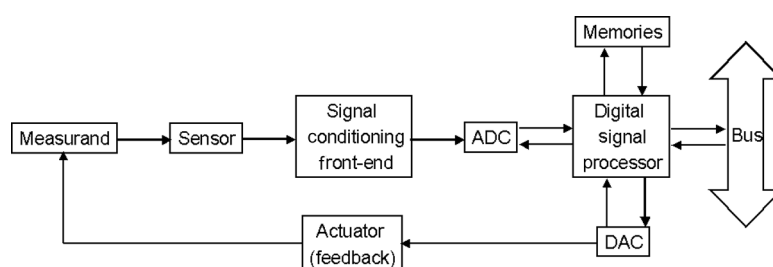


Fig. 1. Essential dataflow functionality of lab-on-chip devices. It can be realized with electronics, incorporated either in the microfluidic chip or on separate circuit boards, attachable to the chip. The sensor is followed by an analogue front-end, which conditions the measuring signal, analogue-to-digital converters (ADC), and a digital signal processor that analyses the signal. Depending on the measuring method the signals can be electrical, optical, etc. The analysed data can be further sent via a bus to external computer for post-processing, or even visualized on integrated displays or external screen. Additionally to processing measurement data, the processor may adjust the state of the measurand sample via mechanical, electrical, or optical actuation.

Lab-on-chip technology has adopted most analytical chemistry methods, such as electrochemical sensing [7,8], mass spectrometry [9,10], thermal detection, ultrasound waves [11], capillary electrophoresis [12-17], electrochromatography [18-24], and moreover optical methods like absorbance [25,26], infrared and Raman spectroscopy [27], scattering [28], refractive index [29], surface plasmon resonance [30], plasma emission and fluorescence [31].

2. ADVANTAGES OF LAB-ON-CHIP DEVICES

Built to automate laboratory processes, some notable technical advantages of lab-on-chip devices are compactness, portability, modularity, reconfigurability, embedded computing, automated sample handling, low electronic noise, limited power consumption and straightforward integration of their components. In addition to minimal quantity request for samples, analytes, or reagents, the lab-on-chip devices are fully enclosed and this reduces contamination of the carried samples. Furthermore, lab-on-chip devices are capable of supporting a wide range of processes such as sampling, routing, transport, dispensing and mixing, mostly with reduced moving or spinning components, therefore the usability and lifespan of the device is increased. Due to their small size, the lab-on-chip devices offer precise fluidic transportation via the use of electrokinetics or micro-pumping, efficient separation of the liquid samples and precision in the measurement of samples.

Although usually the fluidic transportation pertains continuous flows, droplet-based segmented flows are also favoured [32-34]. The droplets usually find applications as small bioreactors for cellular and molecular assays for advanced therapeutics [35-37]. Supplementary, and depending on the particularity of the application, tiny droplets can be employed as electrical [38], optical [39,40] or thermal agents. The benefit for biomedicine is the possibility for continuous and automated monitoring of biochemical samples and the possibility of adjusting procedures by online feedbacks. Due to the small fluidic volumes that they handle, the lab-on-chip devices can reduce the time of synthesis of a product and the time of analysis of a sample. They can measure samples with greater precision, but most essential is their capability of controlling the chemical reactions through efficient control of the reactants concentration. The lab-on-chip devices can either provide cascade or parallel sample processing. The advantage of parallel processing allows simultaneous tests of different samples with different reagents, so that the product's effectiveness can be characterized efficiently.

Lab-on-chip technology offers affordable and simple maintenance of the fluidic chips. They can be easily cleaned and sterilized with cleaning solutions like sodium hydroxide, nitric acid, decanol, ethanol, bleach and ethylene oxide [1]. Alternatively, ultraviolet radiation, autoclaving and heat can sterilize the devices. Furthermore, capillary plasma can remove organic remains from inside the fluidic chips.

Key manufacturing advantages that make lab-on-chip devices affordable are: achievable mass production, affordable replacement cost, short time manufacture, simple quality tests, and broad range of supporting computer aided design and simulation software tools. Most of the advantages of the fluidic lab-on-chip devices are analogous to those of the integrated electronic chips.

3. APPLICATIONS OF LAB-ON-CHIP DEVICES

Clinical medicine greatly benefits from lab-on-chip technology as it suites for drug tests, tests for observing pandemics, glucose monitoring, diabetic control, diagnosis of diseases and numerous other tests. Lab-on-chip devices enhance numerous biomedical tests that entail mixing, analysis and separation of samples, which usually consist of cell suspensions, nucleic acids, proteins, etc. Analytical, electrical, or optical detection methods are possible. The electrical detection methods depend exclusively on the polar properties of the molecules of the liquid samples. For example, carbon dioxide levels, oxygen levels, or pH values can be measured electrochemically. On the contrary, most analytical or optical techniques require labelling, which entails chemoluminescence, fluorescence, or radioactive markers. Most separation methods of lab-on-chip systems are miniaturized approaches of larger ones. There are diverse screening methods, which offer high sample throughput, whereas other methods offer reliability and precision. The separation of biomolecules, cells, or nanoparticles can be managed by transportation methods, which are based on the charge and size of the substances. These transportation methods can be the following ones:

- (a) hydrodynamic manipulation, which employs hydrodynamic pressure [^{41–44}];
- (b) electrical manipulation which transports electrolytes, suspensions of particles or cells, or entire aqueous volumes like droplets, via the use of electrokinetic mechanisms such as electrophoresis [⁴⁵], dielectrophoresis [^{46,47}] and electro-wetting [^{48–51}];
- (c) magnetophoresis that employs magnetic fields in conjunction with magnetic nanoparticles suspended in the samples [⁵²];
- (d) optical manipulation that employs laser-light pressure which moves nanoparticles or tinny droplets [^{29,33}]; alternatively, light actuation can alter locally the degree of hydrophobicity of a surface and consequently direct aqueous volumes via hydrophilic routes.

Electrical and mechanical micropumps are largely employed in microfluidic manipulation [⁵³]. Electric micropumps utilize electrokinetics [⁵⁴], piezo-electrics [^{55,56}], or magnetohydrodynamics [⁵⁷], while mechanical micropumps utilize hydrodynamic pressure [⁵⁸], thermal expansion [⁵⁹], osmotic pressure [⁶⁰], or other transducer or induced forces. Electrocapillary, an important electrokinetic pumping mechanism, boosted the development of lab-on-chip devices [^{13,61,62}]. This achievement caused the realization of miniaturized analytical instruments, namely on-chip chromatographic systems [⁶³]. Other pumping

mechanisms employ thermal gradients, or magnetophoresis. Fluidic lab-on-chip devices can facilitate cell screening [^{64–66}]. This works on the basis of forcing cells into specific streamlines, separates them by size, and drives them into specific outlets [⁶⁷]. This finds applications in blood cell separation, where erythrocytes flow right in the centre of a microcapillary and the leukocytes, due to their differing mass, pursue streamlines along the capillary wall [^{43,68}]. Besides hydrodynamic separation, microvalves can sort suspensions of cells or nanoparticles, usually in conjunction with electrokinetic actuation methods [^{69–71}]. Electrically actuated microvalves are made of electroactive elastomers or piezoelectrics, which can be embedded easily in fluidic chips. Piezoelectric micro-pumps are activated when voltage applies on a piezoelectric material causing expansion. The expansion can be several micrometres, but in the microscale this causes significant pressure, which pushes the fluid adequately. Magnetically actuated microvalves also exist, made of magnetically inductive materials. The magnetic actuation field can be produced on-chip by means of current patterns, integrated on the chip. Lab-on-chip devices are efficient in mixing samples in a controllable and precise manner due to the use of tiny liquid volumes. Examples of mixers include distributive mixers, static mixers, T-junction mixers and vortex mixers. Active mixers consume power in order to increase the interfacial area between the mixing fluids. Examples of active mixers include electrokinetic mixers, chaotic advection mixers and magnetically driven mixers. Passive mixing can be achieved in microchannels with structures called twists that cause turbulence. Some mixers are more efficient at faster flow rates, whereas others work more efficiently at slower flow rates [³²].

Temperature control is important in microfluidic devices. Temperature gradients can be generated inside microfluidic channels by means of miniaturized heating elements, such as heat exchangers, heaters or coolers [⁷²]. The efficiency of micro-heat exchangers depends on their ability to regulate the temperature of the transported fluid, in conjunction with reservoirs that buffer rapid changes of temperature. The micro-heaters are assembled in the form of coils. Due to the inherently small dimensions of the microfluidic devices, the thermal coupling between a micro-heater and a reservoir is very efficient. Coolers can be made out of heatsinks that induct the heat to the environment. The combination of small fluidic volumes and the precision of the heatsinks allow cooling to ambient temperature with ease. Repeatable temperature cycles are essential in the process for nucleic acid amplification and find application in the polymerase chain reaction (PCR) arrays-on-chips [^{72–77}]. PCR replicates small amounts of nucleic acids into large amounts. PCR involves a three-step thermal cycle that promotes the replication: heating (90–95°C), cooling (50°C), and slight warming (60–70°C). PCR chips include various temperature zones whose benefit is that the cycle time no longer relies on the time required to heat or cool the sample. The benefit of performing PCR in microfluidic chips is due to the very small liquid volumes used, for which temperature homogeneity and diffusion efficiency are higher in comparison with ordinary laboratory vessels. However, thermal cycles

may induce undesirable effects, especially regarding evaporation and bubble formation. The droplet-based biochips eliminate most of these defects during PCR processes due to the isolation of the droplets by a second phase oleic fluid [^{78,79}]. Fully automated PCR biochips that contain mixers, heaters, and microarray sensors are capable of performing sequential functions like hybridization, preconcentration, purification, lysis, and electrochemical assessment of complex biological solutions [⁸⁰]. In bio-analysis, biosensors, integrated in microfluidic devices, offer possibilities for electrochemical or optical analysis of samples. Enzyme-based electrochemical biosensors can measure oxygen consumption or pH production by means of enzymatic reactions. The enzymes catalyse the reactions of the analytes and the sensor measures the products [⁸¹]. In the field of bioanalytical separation, the capillary chromatographic chips adopt fluorescent polystyrene nanoparticles or labelled macromolecules to enhance cell or protein separation by image recognition [^{18,82,83}]. In analogy, the ion exchange chromatographic chips separate electrokinetically analytes such as polar molecules, proteins, nucleotides and amino acids according to their ionic charge. Genomic research requires analysis, which can be utilized by means of specialized fluidic chips called microarrays, which are arrangements of open planar arrays that each contains thousands of specific oligonucleotides, covalently bonded on glass or silicon substrate [⁸⁴⁻⁸⁷]. These oligonucleotides function as probes, which hybridize the DNA fragments that contact the microarray, by forming hydrogen bonds between the DNA fragments and the complementary nucleotide base pairs of each probe. A microarray can accomplish many genotype tests in parallel. The detection method in microarray chips is based on microscopy analysis via the use of fluorophore or chemoluminescence labels that determine abundance of nucleic acid sequences. The lab-on-chip microarrays can be distinguished between cDNA-microarrays and oligonucleotide-microarrays. The latter can perform genotyping and resequencing [⁸⁸]. The microarray chips can be employed in nucleic acid analysis, which includes DNA extraction and purification, amplification, hybridization, sequencing, gene expression analysis, genotyping and DNA separation. Moreover, microarrays can recombine nucleotide fragments by employing endonucleases to cleave and rebind the fragments with DNA ligase enzymes. Fluorescence microscopy is mainly employed in microarrays because of its challenging limits of detection, due to its sensitivity, which approaches concentrations of picomoles. Detection sensitivity of picomoles is appropriate in most applications that require high sensitivity, such as DNA sequencing and many immunoassays [⁸⁹]. There are great potential applications of microarray chips in biotechnology as well as of DNA-inspired lab-on-chips in nanotechnology [⁹⁰]. The oxidative type of DNA-mediated charge transport has significant results in mutagenesis, apoptosis, or cancer studies. On the other hand, excess electron transport plays a growing role in the development of electrochemical DNA chips for the detection of DNA base mutations [⁹¹]. Knowledge about excess electron transport in DNA has potentials for nanotechnological applications, such as supramolecular electronics. In this field, the

research focuses on the role of DNA as supramolecule that features important properties for nanowires [92].

Lab-on-chip devices support cell detection by means of capillary electrophoresis, electroporation, cytometry or electrical impedance. The cells can be cultivated within aqueous microreactors inside the chips [35,69,70,93]. With lab-on-chip devices one can measure accurately chemical stimuli on cells, as cellular signals are weak and not easily detected with conventional analytical methods. Cytometers allows cell counting and analysis of cellular parameters like size distribution or growth rate. Cytometry commonly employs electrical impedance methods [94–96], but also employs fluorescence microscopy; however, fluorescence microscopy requires cell labelling with dyes or fluorescent nanoparticles and external optical apparatuses, such as optical fibres and illuminators [97]. Other electrokinetic methods rely on contactless conductivity detection of organic ions or electrolytes where typically two on-chip embedded tubular electrodes, away from each other, measure changes of the ohmic resistance of the solution that flows inside the microfluidic channel [8,15,17,98–101]. Capillary electrophoresis performs electroseparation of the ionic compounds, based on the size-to-charge ratio of the ions, which are transported electrokinetically in the carrier liquid. The electrocapillary forces drag the ions into the capillary channel while the fluidic sample approaches the inlet of the microchannel [102–104]. Capillary electrophoresis is capable of separating particulate substances like cells, amino acids, proteins, peptides, mitochondria, or bacteria [105]. In microfluidic channels the surface-to-volume increases significantly due to reduced dimensions, and electrophoresis force becomes more efficient due to the shortening of the distance. In capillary electrophoresis devices the electric fields range to some hundreds V/cm with pulse durations of milliseconds. Capillary electrophoresis separation is fast and robust and high throughput can be obtained. Electroporation is an electrical method that accesses the interior of cells. Applying short and intense electric pulses increases the conductivity and transiently permeabilizes the lipid membrane of a cell and introduces substances into the cytoplasm. Electroporation is used for inserting drugs or genes into cells and is highly efficient when performed *in vitro* in lab-on-chip devices [106]. Electrokinetic manipulation of suspended particles by means of electric fields is widely used in lab-on-chip devices for *in vitro* separation of particles or cells [107,108]. Dielectrophoresis manipulates and separates cells, beads or nanoparticles by means of inhomogeneous electric fields that can be produced with electrodes of specific shapes [46,47]. The cells can be collected or directed away from the electrodes under the influence of the dielectrophoresis force, which is directed towards the field gradient, and originates from the permittivity difference between the carrier fluid and cells [109]. The possibility of using dielectrophoresis for *in vitro* separation of blood cells, based on their conductivity characteristics, can automate partition of white blood cells from erythrocytes. Several human cancer cells have been successfully studied and dielectrophoretically sorted [110–113]. Dielectrophoresis can further measure dielectric differences of cells or particles by means

of electrorotational spectra [114]. Cancerous cells are different from healthy erythrocytes and lymphocytes. Their dielectric diversification can be exploited for removing metastatic cancer cells from healthy blood cells. Other electrokinetic means with growing applications in lab-on-chips technology is electroosmotic flow, a coulomb effect that results in transportation of ions of an electric double layer [115,116], and its reverse effect, streaming potential, that is induced voltage on an electrode due to the flowing motion of counterions of an electric double layer [117,118].

Today, magnetic separation devices are available and magnetic cell separation offers diagnostic and therapeutic values [119]. Magnetophoretic sorters can capture cells that are bonded with magnetic beads, or nanoparticle aggregates, and force them flow into the carrier fluid with increased selectivity [52,120]. Moreover, magnetic nanoparticles can be used to destruct targeted cells by penetration, or to release carried drug into the cell's cytoplasm [121]. The magnetic field can be generated by current patterns on the chip. An important advantage of magnetic actuators in comparison with the electric ones is due to the magnitude of the magnetic driving force that is significantly larger in comparison to electrokinetic forces. This highly improves the control of the liquid flow. The possibility of incorporating magnetic nanoparticles into cells as inert tracers for cell monitoring, allows measuring cytoskeleton associated cell functions [122,123]. Within cytoplasm the magnetic nanoparticles may equilibrate, but intracellular transports disorient the magnetic nanoparticles resulting in the decay of magnetization which can be recorded by magneto-impedimetric sensor, embedded in the chip. Magnetic actuators can transport aqueous droplets that enclose magnetic nanoparticles [124]. Under the influence of the magnetic field the magnetic nanoparticles can drag the droplet and execute all the usual microfluidic operations such as displacement, merging, mixing and separation [125].

4. DESIGNING AND MANUFACTURING PROCESS

The manufacturing process for producing a lab-on-chip device is described in Fig. 2. A numerical simulation that models the performance of a lab-on-chip device is essential before manufacturing the device. Simulations are important,

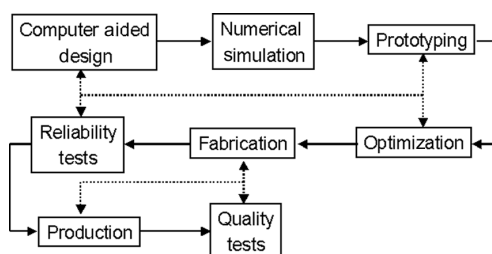


Fig. 2. Flow diagram of the manufacturing steps required for producing a commercial lab-on-chip device.

because they imitate the functionality of the components of the device and then use the simulation data to infer the real-world functionality and performance. Numerical simulations usually rely on finite element algorithms that can be actualized by specialized finite element software. Fully dynamic simulations are computationally not efficient, because they require huge computational resources. Alternatively, the numerical simulations can be performed quasi-statically, but this does not provide information about the dynamic behaviour of a component. In order to study the dynamics of a component in a computationally efficient way, it is essential to reduce the number of degrees of freedom, from a three-dimensional model to a two-dimensional model. Due to the hierarchical structure of lab-on-chip devices, a numerical simulation requires studies of scalability [2]. A lab-on-chip maker must handle heterogeneous and multiple components and should deal with complex fluidic flows and multiple component simulation. It is important for the chipmaker to investigate how the performance of a microfluidic device scales with increasingly complicated sample analysis. In addition, it is necessary to investigate how the performance of the lab-on-chip system balances with advances in the present technology. Although a number of computer-aided design tools, dedicated for lab-on-chip designs, exist, any usual computer-aided design tool that supports multilayer drawing and vector graphics can be used.

Lab-on-chips devices should be modular to allow, to the most possible extent, expansion to varied functions. The goal is to leverage system-level and component-level technology, including fluidics and sensorics, to be expanded, reconfigured and reused for varieties of applications on the same chip. Usually data analysis software can be reconfigured. For example, a lab-on-chip microarray device should be capable of processing genotyping and gene expression applications. A chromatographic fluidic biosensor or a temperature-based actuator should be capable of processing liquids and gases. An electrokinetic actuator should be capable of processing both continuous or segmented fluidic samples and the same device be competent of separating nanometer-size macromolecules by means of electrophoresis, or separating micrometer-size cells by means of dielectrophoresis, or separating millimetre-scaled droplets that enclose cells, by means of electrowetting. Segmented flows that consist of droplet microreactors allow dynamic reconfigurability, because the cells, cultured inside each droplet, can be reconfigured to different functionality within each individual droplet.

The system architecture of a microfluidic chip should assort the elementary fluidic components like micropumps, microvalves, reservoirs, microchannels, nozzles and junctions in a manner that allows a functional and straightforward process. It is convenient to consider circuit analogies when analysing microfluidic lab-on-chips: the reservoirs are comparable to registers as they are storage components. Microchannels are equivalent to wires as they carry flows. Microvalves are analogous to gates as they provide selectivity. Micropumps are analogous to voltage sources as they actuate the flow.

Electromagnetic compatibility issues should be carefully considered in lab-on-chip design because measuring and control signals usually interfere. To eliminate electromagnetic interferences, usually timeslot sharing is required. Furthermore, the metal holder of a lab-on-chip should be designed in a way that shields the device. The electronics and embedded circuits that integrate onto a lab-on-chip device should comply with the electromagnetic compatibility standards of radio frequency electronics. Any circuit board, attached on the chip holder, should be designed in a way that minimizes electrical connections. This is particularly important in the impedimetric lab-on-chip devices, which operate at radio frequencies, where the electric length of the connections influences the measurement of the impedance values.

The lab-on-chip devices should be designed in a manner to be easily maintained. A device should offer easy channel washing and sample filling. The chip holders and the other connecting components should be easily handled, assembled and used by the laboratory staff. Many lab-on-chip devices might be requested to fulfil specific customized tasks, but still the manufacturer should design the device in a manner that provides the user the fundamentals for handy maintenance, calibration and operation.

The manufacturing procedure for producing lab-on-chip devices is comparable to the microelectronic chip fabrication procedure. It involves photolithography, where patterns that represent microfluidic and electric features are transferred onto a photosensitive substrate by means of ultraviolet illumination via a chromium photomask. Then chemical etching reveals the elements. Application of photolithography via successive photomasks produces multi-layered structures. Photolithography has continuous improvements in the ability of resolving ever-smaller features, but also in producing high-aspect-ratio features. This is especially important in the fabrication of microfluidic channels. Each implementation of the photolithographic process has its own specific requirements, but there is common sequence, which involves the following, as shown in Fig. 3.

- (a) Metallization of the substrate by sputtering gold or platinum film on it. Electrodes and other conductive elements can be shaped on this metallized surface.
- (b) Spin-coating of photoresist onto the metallized surface, at typical speeds (depending on the resist's viscosity) of 1500–8000 rpm, and heat at 100°C to dry the resist. The photoresist serves as an intermediate layer via which the patterns can be transferred onto the metallized substrate.
- (c) Exposure of the photoresist layer to ultraviolet light that results in the transfer of the patterns onto the photoresist film via photomasks. The resist's solubility alters on the exposed areas.
- (d) Dissolution of the exposed areas of the photoresist with ammonium hydroxide.
- (e) Chemical etching that removes the metal that extends under the exposed photoresist. Dissolution of the unexposed areas of the photoresist in order to

reveal the metal patterns, which extend underneath. It follows plasma treatment that removes the organic remains and cleans up the substrate. (Alternative to chemical etching is the lift-off process, where metal sputters the top of a photoresist film and covers only the uncoated areas of the substrate. It follows detachment of the unexposed areas from the substrate.)

- (f) Spin-coat SU8 photoresist, at typical speed of 1500 rpm for 30 s, and hardening at 90°C for 2 min. The SU8 layer may develop up to thicknesses of some hundred micrometers. This SU8 layer is used as a material for producing microchannels.
- (g) Transferral of patterns onto the SU8 layer by ultraviolet exposure of the SU8 via chromium photomask. The SU8 resist is then developed with methoxypropyl-acetate that removes the unexposed SU8 areas and reveals the microchannels. (Alternative to SU8, microchannels might be directly etched on glass substrate by means of hydrofluoric acid etcher.)
- (h) Dishing of the substrate in order to separate the individual chips and drill of inlets.
- (i) Enclosure of the chip by aligning and crosslinking two opposite plates at the temperature 150°C for 30 min.

Soft lithography is an alternative fabrication technique, based on direct printing or molding of polymers [126,127]. In soft lithography, instead of stiff photomasks, elastomers are used as stamps, molds, or masks, in order to replicate patterns (Fig. 4). In soft lithography the silicone elastomer PDMS (polydimethyl-

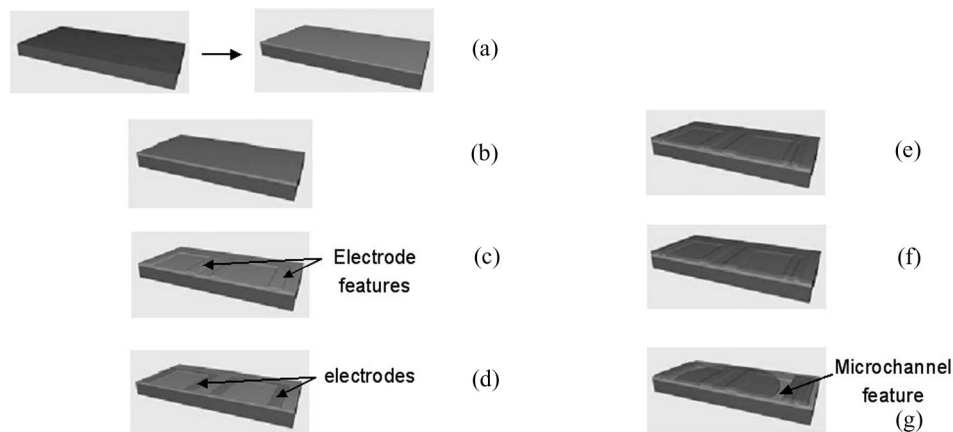


Fig. 3. Photolithographic development of microelectrodes and microchannels on a wafer substrate: (a) metallization of the substrate by sputtering a metal film of Au, Pt, or ITO; (b) spin coating of photosensitive resist film onto the metal film; (c) exposure of the photosensitive film via a photomask that results in the transfer of the desired electrode patterns onto the photosensitive film; (d) after photo-development, chemical etching removes the bare metallized areas, which results in the formation of the electrodes; (e) spin coating of an SU8 photoresist layer; (f) exposure of the SU8 photoresist film via photomasks and development; (g) chemical etching and removal of the unwanted SU8 resist. The revealed half microchannel has sidewalls made of the SU8 material.

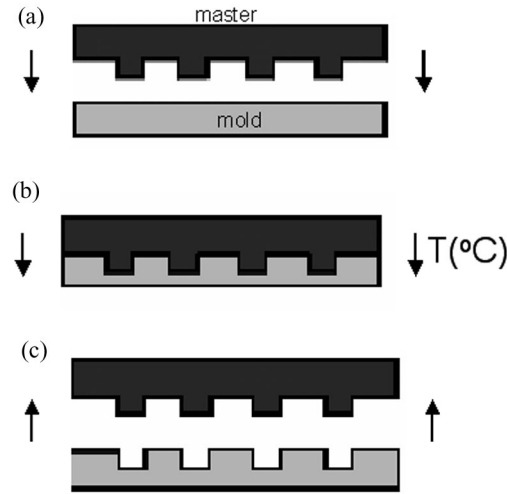


Fig. 4. Sequential steps of the soft fabrication process, which can produce features of a few hundreds of microns: (a) a hard master substrate made of metal, glass or plastic, casts on a mold material which can be polymer, thermoplastic, PDMS or PMMA; (b) upon heating and pressurization the mold deforms to the shape of the master; (c) lifting off the master reveals the microchannels.

siloxane) is widely preferred because of its easiness to cast, its biocompatibility, hydrophobicity, and sealing properties. Soft lithography is recommended only for rapid prototyping in research. Because of the soft materials used in soft lithography, distortions of stamps and molds are problems that prevent soft lithography from becoming a viable manufacturing technique. For example, the widespread PDMS swells when it comes in contact with oleic solvents.

5. MATERIALS AND METHODS

5.1. Fabrication materials

Lab-on-chip fabrication techniques are analogous to those of microelectronics, since closely related microfabrication and integration methodologies are shared by both. The very first fluidic devices were fabricated on silicon (Si) substrates by means of silicon integration technology and afterwards a more advantageous material, glass (SiO_2), was introduced that demonstrates robustness and better electrical insulation [128–132]. Modern lab-on-chip devices are hybrids that combine glass, silicon and various polymers like acrylic, polyester, polycarbonate, resists, thermoplastics or molds like the polydimethylsiloxane (PDMS). Precision, miniaturization, cost effectiveness, large-scale production and ability of incorporating electronics make these materials very attractive. They are proven very reliable since they demonstrate strong crystalline structures and can survive long-term use.

Silicon, glass or polymers are suitable for making the microfluidic components of the chips; metals like gold, platinum or titanium are used for the conductive parts; silicon dioxide, silicon nitride and titanium nitride are for insulation and passivation; electroactive polymers or other elastomers are for the moving components. The standard procedure of producing fluidic chips on silicon or glass substrates involves:

- (a) deposition of metallic and dielectric layers on silicon or glass wafer;
- (b) patterning these layers by means of photolithography in order to transfer onto them the outlines of the microchannels, conductive or insulated areas, or other features;
- (c) chemical etching that removes the unwanted material and reveals the desired structures.

Specifically for capillary electrophoresis devices, the use of silicon substrates is limited by their semiconducting property; due to the high electric fields required for performing capillary electrophoresis, electric shorting very likely occurs. For this reason, glass substrates are mostly preferred for making capillary electrophoresis devices.

Polymers, like polycarbonates, have varied interfacial and structural characteristics. Polymers have disadvantageous optical properties in comparison to glass, like lower light transmittance and higher self-fluorescence emission, but better thermal conductance. Microfluidic channels can be made of polymers by means of molding or micromachining [^{133,134}]. Particularly fluoropolymers, such as polytetrafluoroethylene (PTFE (C₂F₄)_n) are handy materials for structuring microfluidic channels as they are soft and can be easily milled, and are also hydrophobic, which highly eases liquids to flow within the microchannels. For rapid prototyping, casting of polydimethylsiloxane (PDMS (C₂H₆OSi)_n), by means of silicon or smoothed metallic masters, can produce PDMS microfluidic channels [¹³⁵]. PDMS provides flexibility, hydrophobicity and very tight sealing under application of uniform mechanical pressure. PDMS is capable of sealing various smooth surfaces, including glass, silicon, silicon nitride, polyethylene, glassy carbon, oxidized polystyrene, fluorocarbons and metals. Since soft lithography is usable for fast prototyping, PDMS is preferred in soft lithography as an easy-to-cast material for outlining microchannels on smoothed substrates. Compared to glass, PDMS has lower thermal conductivity, much higher hydrophobicity, and both these properties qualify PDMS as a suitable material for making microfluidic channels. On the other hand, PDMS is swollen by many organic solvents like oils, but remains unaffected by water, nitromethane, ethylene glycol, acetonitrile, perfluorotributylamine, perfluorodecalin and propylene carbonate. Furthermore, PDMS is known to absorb small lipophilic molecules [¹³⁶]. The compatibility of PDMS against organic solvents can be enhanced by means of coating the PDMS surface with sodium silicate. Yet, due to the pliability of PDMS, electrodes cannot be patterned on it [¹].

Metal films like gold (Au), platinum (Pt) and titanium (Ti), or metalloid films like Indium Tin Oxide (ITO), are appropriate for making microelectrodes for lab-

on-chip devices. Gold and platinum are highly biocompatible, chemically inert and thus are suitable metals for contacting biochemical solutions. Thin gold, platinum, titanium, or ITO films may be sputtered, or deposited by means of evaporation, on wafer substrates. The electrode patterns subsequently can be revealed by chemical etching or lift-off process, which allows resolution of few micrometers. The chemical etching method is analysed below in Section 5.4. Alternative to chemicals, it is possible to reveal electrodes by means of precise milling of the metallized substrate at resolution of few tens to hundreds of micrometers. The electrodes of microfluidic devices are usually coated with a dielectric film because the dielectric coating prevents electrolysis of the contacting fluids. But in the case of very low voltage applications, such as bio-impedance, the electrodes can remain bare. Other usages of metal films in lab-on-chips technology entail production of masks, overlayers, or adhesion layers. Yet, microfluidic heaters or heat elements of Pt/Ti can be structured with 200 nm Pt, electrodeposited onto 20 nm Ti film. Pt thin films on glass substrate are capable of withstanding thermal bonding and thus Pt is preferred for wiring contacts [1].

For controlling liquid flows, movable micro-apparatuses might be integrated within lab-on-chip devices. The electroactive polymers are an important class of elastomers for fabricating microvalves or micropumps, as they can be precisely controlled electrically.

5.2. Coating materials

The metallization of silicon, glass or polymer substrates is a process which precedes the patterning of the electrodes. Metal films can be sputtered or chemically deposited on silicon, glass or polymer substrates. Gold and platinum are two metals widely adopted by the chipmakers in the production of micro-electrodes, due to two significant properties:

- (a) excellent conductivity, which allows transmission of electrical signals without distortions;
- (b) biocompatibility, which allows direct contact with biochemical solutions without adhesions and adsorptions.

The density of a metal film that is developed by means of chemical deposition is a bit lower than that of bulk metals. The deposited metal film might contain defects such as pores and inclusions of foreign matter of 2–30 nm in diameter. Further, its mechanical strength depends on its chemical composition, deposition rate and temperature and pressure conditions. For instance, mechanical strengths of 40–50 kg/mm² might be obtained at 50–70°C [137]. The electrical conductivity of chemically deposited metal films is usually lower than that of pure metals. The optical properties, however, are less varied and do not differ much of those of pure metals. The optical characteristics of a fabrication material should be considered in those lab-on-chip devices that work with optical sensing apparatuses. In the case when a microfluidic device integrates optical sensors, the microfluidic channels of this device should be made transparent in order not to disrupt the

light beam. Transparent electrodes like indium-tin-oxide might be required in the case when electrodes and optical mechanisms are combined on the same microfluidic channel.

Yet, it is possible to develop gold electrode patterns of micrometer thickness on polymer substrates by means of aerosol spraying metallization, where gold and amines, combined together with hydrazine as reducer, produce gold films of thickness of 400 nm/min [¹³⁷]. Similarly, platinum can be deposited adjustably at rates of 500–2000 nm/h with borohydride or hydrazine as reducing agents.

Oxide films of nanometer thickness are usable as electrode insulators, because they prevent hydrolysis of fluids. It is possible to accurately grow oxide layers on electrodes by means of deposition, sputtering, or anodic or thermal oxidation. The oxide films further protect the electrodes from chemical aggressions, especially those electrodes that come to direct contact with electrolytes, biological solutions or acids. An oxide protective film prevents electrolysis because of high resist against leakage currents, produced by high-voltage electrokinetic phenomena in fluids (e.g. electrophoresis, dielectrophoresis, electro-wetting). However, for low voltage impedimetric applications, mV or below, it is feasible to directly contact the liquid sample with bare electrodes. Thermally grown oxide films, like silicon dioxide (SiO_2) and the self-healing tantalum pentoxide (Ta_2O_5), are appropriate options for robust electric insulation due to their high breakdown resistance [^{51,138,139}]. Thermally grown SiO_2 layers may form thicknesses of some nanometers. SiO_2 and Ta_2O_5 can, alternatively, be deposited by plasma, or be sputtered on metal electrodes. However, compared to the deposited ones, the anodically or thermally grown oxide nanofilms are more effectual in terms of adhesion and electric insulation, due to their layer's continuity with the crystalline structure of the metal electrode and their low pinhole density.

Ceramic coating films, like silicon nitride (Si_3N_4), can be deposited by means of chemical vapor deposition, or plasma-enhanced chemical vapor deposition. Si_3N_4 films are used as electric insulators and are superior to SiO_2 , but weaker than Ta_2O_5 . Strontium titanate (SrTiO_3) has very large permittivity and thus it is suitable for high voltage electric insulation.

Transparent electrodes, like Indium Tin Oxide, are favoured in lab-on-chip manufacturing, because their transparency allows microscopy. Indium Tin Oxide films are mostly deposited on substrates by means of electron beam evaporation, physical vapor deposition or sputtering.

The hydrophobization of microfluidic channels enables repulsion of aqueous fluids and improves their flow, especially the droplet-based segmented flows. Hydrophobic films are very suitable as coatings for microchannels. Different hydrophobization methods have been introduced, ranging from spontaneous covalent bonding of hydrophobic self-assemblies of organometallic octadecyltrichlorosilanes, a process called silanization, to chemical and plasma deposition of fluoropolymers [^{140–146}]. Alternatively, instead of hydrophobizing a microchannel, it is possible by introducing hydrophobic surfactant into aqueous droplets to

encapsulate them into surfactant membranes. Supreme surfactants are the fluoro-surfactants $(C_nF_{2n+1})_n$ [¹⁴⁷].

Prior to coating processes, some chemical treatment in the interior of a microchannel can enhance the adhesion of a coating material onto the microchannel. For example, a glass microchannel can be cleaned with ethanol, acetone, or a mixture of strong acids. Likewise, in polymeric microchannels the adhesion of a film develops faster on prepared and cleaned surfaces and this results in lasting coatings. Some molded thermoplastics, processed in high melt temperature, followed by rapid cooling, are easier to coat because their surface activity increases adhesion. Plasma treatment also cleans organic matter from the interior of a microfluidic chip. Plasma cleaning can either be performed before assembling the chip or afterwards. In the latter case capillary plasma can be produced within the fluidic channels by following the steps:

- (a) seal the microchannel inlets with needle electrodes,
- (b) pump out the air from the microchannel in order to generate vacuum,
- (c) apply high voltage pulses, of few thousands of volts, to initiate electric sparks, which rapidly evolve into plasma arc, which can become visible through the transparent microchannel of the chip. The plasma can be produced with reaction gases such as neon, helium and argon.

Treatment using strong acids can functionalize the inner surface of a microfluidic channel. For example, introducing nitric acid into a glass microchannel it can functionalize the hydroxyl groups of the glass to strengthen the bond between octadecyltrichlorosilanes and the glass and consequently produce a uniform hydrophobic thin layer inside the microchannel.

5.3. Deposition methods

Chemical vapour deposition is a process that produces thin metal, ceramic, or compound films, through thermal oxidation in a gas chamber at an elevated temperature. Within the chamber the substrate interacts, at temperatures between 800–2000 °C and pressures between millitorrs to torrs, with volatile precursors that react and decompose on the substrate a film of a metal (e.g. Al, Ta, Ti, Pt), ceramic (e.g. Si_3N_4 , B_2O_3 , BN) or compound. The gas mixture typically consists of reducing gas, like hydrogen (H_2), inert gases like nitrogen (N_2) or argon (Ar), and reactive gases such as metal halides and hydrocarbons. A typical chemical reaction sequence includes pyrolysis, reduction, oxidation, hydrolysis and corededuction. For example, silicon nitride (Si_3N_4) can be deposited by means of reaction of dichlorosilane gas ($SiCl_2H_2$) with ammonia gas (NH_3) at temperatures between 700–800 °C [⁷²]. The volatile byproducts can be blown away from the reaction chamber and neutralized before exposure to the environment. The chemical vapour deposition can also be plasma enhanced, a method that functionalizes surfaces and is effective in depositing hydrophobic films on wafers. For example, a fluorocarbon hydrophobic film can be developed on an interface by means of plasma-enhanced chemical vapour deposition of CHF_3 . The plasma-

enhanced technique can also be used in the case of depositing insulating films such as silicon nitride (Si_3N_4) or plasma coated silicon dioxide (SiO_2). A similar method, capillary plasma, produces gas plasma within a microchannel, by sealing its inlets with electrode needles, pumping out the air from an outlet, inserting the reaction gases and applying high voltage pulses across the needles. In this way a plasma arc is produced that can be clearly visible if the channel is transparent. Capillary plasma deposition of platinum bisacetylacetonate precursor is performed for deposition of platinum inside channels. The closely related technique of plasma electrolytic oxidation, or microarc oxidation, can electrochemically produce on top of an electrode an oxide coating of micrometer thickness, which provides electric insulation with excellent adhesion.

Adsorption is a chemical deposition method that exploits hydrophilic head groups of self-assembled monolayers of, for example, hydrophobic trichlorosilanes ($\text{Cl}_3\text{SiC}_n\text{H}_{2n+1}$), or thiols ($\text{HSC}_n\text{H}_{2n+1}$), which offer spontaneous binding on glass or metal surfaces [148]. A glass substrate can be functionalized by acidic treatment with a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2), which is well known for producing silanol groups on glass. Silanes bind covalently to the hydroxyls of silanols, with glass being the most obvious surface with silanol groups.

Physical vapour deposition, such as sputtering or evaporation, is used to deposit thin films, layer by layer, onto substrates. This method employs mechanical or thermodynamic means for producing thin films and requires low-pressure vaporized environment to function.

Ion beam enhanced deposition influences the energy and charge states of the gas in the vapour phase and allows control over the energy state and crystallographic and stoichiometric form of the deposited films.

5.4. Etching procedures

In lab-on-chip fabrication technology, patterning is the transfer of outlines of features (which define microchannels, microelectrodes, or other components) on the top of a substrate by means of ultraviolet illumination via a photomask. The photomask consists of a chromium layer where only part of it is transparent to the light. Photolithography is the method for producing structures by exposing photosensitive resists to ultraviolet light via successive photomasks and then removing the exposed areas of the photoresist in a development process. The photosensitive resist is a polymer, usually epoxy, that is sensitive to ultraviolet light. When the ultraviolet exposes specific areas, the photoresist polymerizes and resists to etchers. This replicates the patterns onto the photoresist film. The exposed areas of the resist film are being attacked by the developer and consequently removed. The unexposed areas remain resistant to the action of the etchant. The structure can be revealed after etching the exposed areas. Every wafer substrate may undergo many sequential etching cycles before completes.

The resolution of the features that can be structured with photolithography can be some micrometers. The major steps of the etching process are shown in Fig. 5.

We distinguish between two etching processes, wet etching and dry etching.

Wet chemical etching is widely used for producing microelectrodes and microfluidic channels on substrates. The wet etching requires acids, bases or mixtures to dissolve metals, silicon or glass, by immersing the substrate into the etching solution. For instance, gold can be etched using iodine solution. The etching can be isotropic or anisotropic. As an example, silicon $\langle 100 \rangle$ can be etched isotropically with HF-HNO_3 , which produces rectangular curved grooves by means of thermally grown SiO_2 mask. KOH can etch $\text{Si} \langle 100 \rangle$ or $\text{Si} \langle 110 \rangle$ anisotropically, with the etching rate depending on the crystallographic orientation, and produces grooves with 55° or 90° walls, respectively. It is important to mention, however, that with anisotropic etching the exact defined shape is difficult to be obtained because of the directional dependence of the etching rate, and so this method is limited to small dimensions only. Glass can be etched isotropically using buffered hydrofluoric acid (HF) in HCl or ultrasonic bath that improves smoothness of the etched surface. Removal rates of glass are typically between $0.1\text{--}1.0\ \mu\text{m}/\text{min}$. The etchant should be selected carefully in order to have the etching rate of the masks considerably lower than that of the removable material. The final step in a wet etching process is to remove the resist by washing it away with an organic solvent.

Dry etching involves reactive ion etching with plasma, where the substrate is placed into a plasma chamber where a gas mixture is introduced and is ionized. The ionized gas mixture reacts with the surface of the substrate to be etched. As the ionized gas is highly energized, it removes the matter from the substrate. Xenon difluoride (XeF_2) is a dry vapour phase isotropic etcher for silicon.

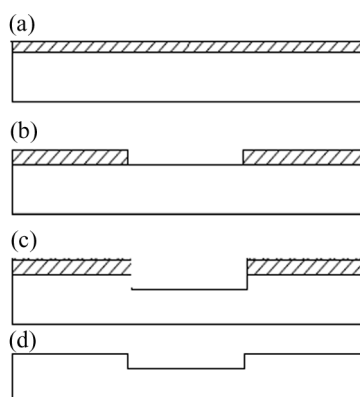


Fig. 5. Sequential steps of a chemical etching process, which can produce microchannels with width of some tens to hundreds of microns: (a) exposure of a photoresist-coated substrate to ultraviolet light; (b) dissolution of the exposed areas of the photoresist with chemical developer; (c) chemical etching of the bare areas of the substrate; (d) revelation of the microchannel after total removal of the photoresist from the substrate.

Its etching selectivity to silicon is very high and does not affect masks made of photoresists, silicon dioxide, silicon nitride or metals. For example, the selectivity to silicon nitride is better than 100:1 and reported to be as high as (5000–10 000):1 for silicon dioxide [¹⁴⁹]. SF₆ in high-density plasma provides anisotropic high-aspect-ratio etching for silicon. Typical dry etching rates for silicon are around 2 µm/min.

A suitable photoresist for structuring fluidic microchannels on wafer substrates is the biocompatible and chemically inert epoxy SU8, which develops vertical sidewalls of micrometer height on glass or silicon wafers [^{150,151}]. The SU8 photoresist can be spun onto the wafer substrate using spin-coater at a speed that achieves specific thickness, under certain viscosity value and specific hydrophobic surface. For example, with usual viscosity and hydrophobicity, to obtain 100 µm thick layer of SU8, the wafer substrate should be spun at 500 rpm for 5 s, to spread out the photoresist, then at 3000 rpm for 30 s, to achieve the final thickness. The substrate should then be heated for 10 min at 65°C, and for 30 min at 95°C. The two-step bake is required in order to reduce internal stresses owing to thermal effects. The photoresist is then exposed in ultraviolet via a photomask. Solidifying one SU8 formation against another by crosslinking the opposing SU8 layers and baking at 65–95°C, it is viable to produce microchannels. The low surface roughness of SU8 enables the use of optical analytical techniques such as fluorescence or optical spectrometry. The possibility of integrating microelectrodes on SU8, the possibility of hydrophobizing the SU8 and of bonding it at low temperature, makes SU8 a particularly suitable resist for assembling lab-on-chip devices. Furthermore, SU8 is suitable of serving as mask on the top of the silicon wafer in wet etching processes [¹].

6. BONDING

After patterning all features on substrates (microchannels, elements, inlets, etc), the base plate and the cover plate must be bonded in order to seal the chip. It is possible to bond silicon, glass, or rigid polymer plates, by three different means.

Anodic bonding can fuse silicon or glass plates. The two opposing plates must come in contact with each other, in heat, with a high voltage applied across a conductive layer (200 nm silicon nitride (Si₃N₄) or 120 nm Ni/Cr) developed intermediately, that causes diffusion of ions, which eventually fuses the two plates electrostatically. Anodic bonding conditions vary between 200–1500 V, at 200–450°C.

Thermal bonding can fuse glass or polymer plates. Thermal bonding is performed on coated with methylsilanes-quiexane, or polysiloxane, surfaces at temperatures 150–210°C. Annealing for some hours in low vacuum produces bonds that withstand hundreds of N/cm². Rapid glass bonding requires hot pressure at 570°C for 10 min under 4.7 N/mm².

Photopolymer adhesives can bond any type of rigid substrates. This method is more tolerant for uneven surfaces since it provides a compressible cushioning layer that seals the chip. The adhesive layer can be benzocyclobutene (C_8H_8) or UV-curable resins of micrometer thickness. It is possible to heat and separate the plates apart and rebond.

To sandwich elastomers, such as PDMS, between plates, mechanical pressure must be applied uniformly. Uniform pressure is usually obtained by squeezing with a metal holder the plates against the elastomer by means of screws. The advantage is the possibility of reopening the chip.

7. FLUIDIC AND ELECTRIC CONNECTIONS

Customized chip holders can support fluidic tube connections and wired electrical connections. Flanged plastic fittings having standard threads can be used for connecting the tube. This connector type uses a metal O-ring, which presses the flanged tip of the tube against the inlet of the chip and seals the inlet tightly after screwing into the respective threads of the chip holder. Another connection method uses injection-molded connectors with internal threads that can withstand pressure of 1 MPa. Furthermore, adhesive or solder-based chip-to-tube connections can tolerate elevated pressures and solvents.

8. BIOCOMPATIBILITY AND DEFECTS

Biocompatibility concerns the safety of the material and its performance to meet some standards in order to avoid harming the biological substances that come in contact with the material of a device. The biocompatibility of lab-on-chip devices came forth with the advent of implantable biochips. Biocompatibility attempts to avoid materials that cause cytotoxicity, sensitization, irritation, genotoxicity, carcinogenicity or other abnormalities^[88]. The biocompatibility of a device concerns only the materials that come into contact with biological substances.

There are numbers of possible defects, which should be considered when designing biomedical lab-on-chip devices. Since most of the microfluidic channels are made hydrophobic, most enzymes, proteins and cells adsorb on hydrophobic areas, an effect called biofouling. Biofouling disorders the microfluidic chips, because it alters their microchannels into hydrophilic, which affects the laminar flow. Biofouling affects sensing elements, such as microelectrodes, which consequently affects the accuracy of the measurements. Silicon, silicon nitride, silicon dioxide, gold, platinum, titanium, SU8 and photoresists are biocompatible and all of them have reduced biofouling and cytotoxicity^[152–154]. Particularly, silicon nitride is used for long-term implantations.

The proteins bovine-serum-albumin (BSA) and casein (phosphoprotein) reduce the adhesion of enzymes and proteins on the interfaces. Important advantage of bovine-serum-albumin and casein is their biocompatibility with most of the proteins.

To prevent adhesion of cells in microfluidic channels, surfactants like poloxamer copolymers can be added inside the channels^[155]. However, poloxamers have shown to incorporate into the cellular membranes, particularly of the cancerous cells, affecting so the microviscosity of the cell membrane.

9. FUTURE DIRECTIONS

New lab-on-chip devices must emerge that demonstrate a positive impact on clinical diagnostics. The trend is to improve sterility, multiple analysis and parallel processing with increased efficiency and speed. Future lab-on-chip devices will consume ultralow power, will be smaller and lighter with more emphasis on the user comfort. Improvements in portability should emphasize easy sample introduction and fluidic connectivity. Communication technologies like wireless networking, information management and advanced user interface, visualization and navigation technologies through adapted screens will be embedded. Standardization will condition device reliability, interoperability, biocompatibility, electromagnetic compatibility, interconnectivity, readouts, weight and size^[156].

The functionality of future lab-on-chip devices is foreseen to improve clinical tests and will be shifted from general-purpose laboratory instruments to strictly personalized devices. To date the lab-on-chip devices are determined by applications in bioanalysis. Additionally to mixing, measuring, sorting or separation, microfluidic lab-on-chips are foreseen to facilitate on-chip supramolecular chemistry, biomedical implants, biomimetics, and hybrid systems incorporated with blood vessels^[157]. Future applications will certainly include nanobio-technologies. Envisions consider nanoscaled channels, where surface effects, rather than volume, dominate liquid behaviour. Chemical synthesis of macromolecules will be actualized molecule-by-molecule, ensuing fully controllable and accurate synthesis of bioproducts, independent of the constraints of statistics and diffusions parameters that rule present chemistry in vessels.

Experts claim that the complexity of lab-on-chip devices is increasing at rates comparable to Moore's law. Most of the present lab-on-chip devices operate with external computing units. The future trend is to develop fully standalone lab-on-chip devices with embedded computing and calibrating capabilities. Further development of microfluidic biocomputers will take place that will use biological macromolecules, such as nucleic acids or proteins, to perform computations including storing, retrieving, and biological data processing through application of biochemical principles^[158]. DNA computation employs massive parallelism inherent in the small scale of molecules to speed up decisions. The hybridization

of nucleic acids can be either exploited to encode strands of nucleotides to clone nucleic acids, or to self-assembly oligonucleotides. Emphasis is given to improving methods for hybridization by surpassing various physicochemical constraints and use DNA to compute in environments where it is uniquely capable of operating, such as smart drug delivery to individual cells [^{159,160}]. Further variations in biocomputing use proteins instead of nucleic acids [¹⁶¹], or DNA hairpin formation [¹⁶²], self-assemblies and cellular computation, in which cells are considered computational elements.

Networking individual lab-on-chip devices is foreseen to boost lab-on-chip functionalities, as information can be exchanged between individual devices via computer servers in hospitals. Experts speak about interconnecting lab-on-chips to the patients' databases for online updating their medical records and to perform classification of the diseases. Networking biochips can benefit telemedicine, where lab-on-chip testers will be capable of transferring test results to doctors entirely remotely. The upcoming generations of lab-on-chip devices will be cabled or wirelessly interconnected in network-centric data links, being synchronized as terminal testers for the same or different patients, located at a distance. In rural and urban areas lab-on-chip technology can contribute to ubiquitous monitoring the diseases, pathogens, or toxic substances in the environment. For example, miniaturized biochemical marine sensors, submerged in seawaters, can sample contaminants in the oceans. The functionality of such environmental biochips is based on various principles including monitoring oxygen levels, cell analysis of phytoplankton, observation of methane, nitrate and phosphates in seawaters [^{163–166}]. Moreover, autonomous integrated biochips may be distributed in public areas, in buildings, airports, subways, parks, stadiums, schools, and stores, to collect biochemical information and detect the onset of major infectious pathogens or record signs of pollutions, either on air, sea or land.

The impact of lab-on-chip devices in everyday life is expected to be analogous and as revolutionary as integrated circuits and microelectronics. Lab-on-chip devices will irrevocably influence medicine, chemistry, biology, biotechnology and bioelectronics.

10. CONCLUSIONS

The most appropriate materials, fabrication techniques and assembling methods for manufacturing lab-on-chip devices were reviewed in this article. Lab-on-chip devices must be made of materials that are biocompatible, chemically inert, reliable and reusable for lifetime. Presently, gold or platinum are most suitable for structuring conductive elements. Fluoropolymers and silanes are the most suitable for hydrophobizing fluid-solid interfaces. Oxide-grown films or plasma-deposited films, particularly the thermally or anodically grown SiO_2 and Ta_2O_5 , are the most efficient for insulating the conductive elements of the lab-on-chip devices.

Highly reliable lab-on-chip devices are still a long way ahead. The complexity and technological problems to be solved are enormous. Major obstacle is the technological limitation of the present micofabrication materials. Until new revolutionary materials are introduced, any tremendous development in comparison to the present state should not be expected. The to date development of lab-on-chip devices is analogous to the state of the electronics before the discovery of semiconductors. However, once new synthetic materials become available, especially new nanomaterials, an enormous boost, analogous to this of microelectronics in the semiconductors era, is anticipated. It means that lab-on-chip designers are awaiting innovations from material science and chemistry to solve reliability, biocompatibility and integration problems. Furthermore, lab-on-chip technology acquires improvements in the device production methods, and is forecasted to acquire specific technologies from physical sciences and other disciplines of engineering, as well as from biology [¹⁶⁷].

Conditio sine qua non of future lab-on-chip device design are the following very specific requirements and standards:

- (a) biocompatibility, robustness, sensitivity, and reliability;
- (b) low power consumption, high degree of automation and integration, and high sample throughput;
- (c) coverage of a broad range of biological samples and measurement parameters.

ACKNOWLEDGEMENTS

Acknowledgements are due to Dr Uwe Pliquet and Dr Brian Cahill for technical discussions. Financial support comes from a European Commission Marie Curie ERG (project PERG06-GA-2009-256305) and an Estonian Science Foundation Mobilitas Grant (project MOJD05).

REFERENCES

1. Li, P. C. H. *Microfluidic Lab-on-a-chip for Chemical and Biological Analysis and Discovery*. CRC Press, 2006.
2. Zhang, T., Chakrabarty, K. and Fair, R. B. *Microelectrofluidic Systems*. CRC Press, 2002.
3. Reyes, D. R., Iossifidis, D., Auroux, P. A. and Manz, P. A. Micro total analysis systems: 1. Introduction, theory, and technology. *Anal. Chem.*, 2002, **74**, 2623–2636.
4. Auroux, A., Reyes, D. R., Iossifidis, D. and Manz, P. A. Micro total analysis systems: 2. Analytical standard operations and applications. *Anal. Chem.*, 2002, **74**, 2637–2652.
5. West, J., Becker, M., Tombrink, S. and Manz, A. Micro total analysis systems: latest achievements. *Anal. Chem.*, 2008, **80**, 4403–4419.
6. Kost, G. J. *Principles and Practice of Point-of-care Testing*. Lippincott Williams and Wilkins, 2002.
7. Rossier, J. S., Roberts, M. A., Ferrigno, R. and Girault, H. H. Electrochemical detection in polymer microchannels. *Anal. Chem.*, 1999, **71**, 4294–4299.

8. Wang, J. and Pumera, M. Dual conductivity/amperometric detection system for microchip capillary electrophoresis. *Anal. Chem.*, 2002, **74**, 5919–5923.
9. Chan, J., Timperman, A. T., Qin, T. and Aebersold, R. Microfabricated polymer devices for automated sample delivery of peptides for analysis by electrospray ionization tandem mass spectrometry. *Anal. Chem.*, 1999, **71**, 4437–4444.
10. Kameoka, J., Craighead, H. G., Zhang, H. and Henion, J. A polymeric microfluidic chip for CE/MS determination of small molecules. *Anal. Chem.*, 2001, **73**, 1935–1941.
11. Hawkes, J. J. and Coakley, W. T. Force field particle filter, combining ultrasound standing waves and laminar flow. *Sens. Actuators B: Chemical*, 2001, **75**, 213–222.
12. Becker, H., Lowack, K. and Manz, A. Planar quartz chips with submicron channels for two-dimensional capillary electrophoresis applications. *J. Micromech. Microeng.*, 1998, **8**, 24–28.
13. Harrison, D. J., Manz, A., Fan, Z., Luedi, H. and Widmer, H. M. Capillary electrophoresis and sample injection systems integrated on a planar glass chip. *Anal. Chem.*, 1992, **64**, 1926–1932.
14. Wang, J., Chen, G. and Muck, A. Movable contactless-conductivity detector for microchip capillary electrophoresis. *Anal. Chem.*, 2003, **75**, 4475–4479.
15. Wang, J., Pumera, M., Collins, G. E. and Mulchandani, A. Measurements of chemical warfare agent degradation products using an electrophoresis microchip with contactless conductivity detector. *Anal. Chem.*, 2002, **74**, 6121–6125.
16. Zalewski, D. R., Schlautmann, S., Schasfoort, R. B. M. and Gardeniers, H. J. G. E. Electrokinetic sorting and collection of fractions for preparative capillary electrophoresis on a chip. *Lab Chip*, 2008, **8**, 801–809.
17. Laugere, F., Guijt, R. M., Bastemeijer, J., van der Steen, G., Berthold, A., Baltussen, E., Sarro, P., van Dedem, G. W. K., Vellekoop, M. and Bossche, A. On-chip contactless four-electrode conductivity detection for capillary electrophoresis devices. *Anal. Chem.*, 2003, **75**, 306–312.
18. Kutter, J. P. Current developments in electrophoretic and chromatographic separation methods on microfabricated devices. *Trends Analyt. Chem.*, 2000, **19**, 352–363.
19. Jindal, R. and Cramer, S. M. On-chip electrochromatography using sol-gel immobilized stationary phase with UV absorbance detection. *J. Chromatogr. A*, 2004, **1044**, 277–285.
20. Pumera, M. Microchip-based electrochromatography: Designs and applications. *Talanta*, 2005, **66**, 1048–1062.
21. Végvári, Á. and Hjertén, S. A hybrid microdevice for electrophoresis and electrochromatography using UV detection. *Electrophoresis*, 2002, **23**, 3479–3486.
22. Ceriotti, L., de Rooij, N. F. and Verpoorte, E. An integrated fritless column for on-chip capillary electrochromatography with conventional stationary phases. *Anal. Chem.*, 2002, **74**, 639–647.
23. Throckmorton, D. J., Shepodd, T. J. and Singh, A. K. Electrochromatography in microchips: Reversed-phase separation of peptides and amino acids using photopatterned rigid polymer monoliths. *Anal. Chem.*, 2002, **74**, 784–789.
24. Ro, K. W., Chang, W.-J., Kim, H., Koo, Y.-M. and Hahn, J. H. Capillary electrochromatography and preconcentration of neutral compounds on poly(dimethylsiloxane) microchips. *Electrophoresis*, 2003, **24**, 3253–3259.
25. Liang, Z., Chiem, N., Ocvirk, G., Tang, T., Fluri, K. and Harrison, D. J. Microfabrication of a planar absorbance and fluorescence cell for integrated capillary electrophoresis devices. *Anal. Chem.*, 1996, **68**, 1040–1046.
26. Zhu, L., Lee, C. S. and DeVoe, D. L. Integrated microfluidic UV absorbance detector with attomol-level sensitivity for BSA. *Lab Chip*, 2006, **6**, 115–120.
27. Cristobal, G., Arbouet, L., Sarrazin, F., Talaga, D., Bruneel, J. L., Joanicot, M. and Servant, L. On-line laser Raman spectroscopic probing of droplets engineered in microfluidic devices. *Lab Chip*, 2006, **6**, 1140–1146.

28. Chen, C. H., Tsai, F., Lien, V., Justis, N. and Lo, Y. H. Scattering-based cytometric detection using integrated arrayed waveguides with microfluidics. *IEEE Photonics Technol. Lett.*, 2007, **19**, 441–443.
29. MacDonald, M. P., Spalding, G. C. and Dholakia, K. Microfluidic sorting in an optical lattice. *Nature*, 2003, **426**, 421–424.
30. Krishnamoorthy, G., Carlen, E. T., Kohlheyer, D., Schasfoort, R. B. M. and van den Berg, A. Integrated electrokinetic sample focusing and surface plasmon resonance imaging system for measuring biomolecular interactions. *Anal. Chem.*, 2009, **81**, 1957–1963.
31. Haab, B. B. and Mathies, R. A. Single-molecule detection of DNA separations in micro-fabricated capillary electrophoresis chips employing focused molecular streams. *Anal. Chem.*, 1999, **71**, 5137–5145.
32. Squires, T. M. and Quake, S. R. Microfluidics: Fluid physics at the nanoliter scale. *Rev. Mod. Phys.*, 2005, **77**, 977–1026.
33. Pamme, N. Continuous flow separations in microfluidic devices. *Lab Chip*, 2007, **7**, 1644–1659.
34. Teh, S. Y., Lin, R., Hung, L. H. and Lee, A. P. Droplet microfluidics. *Lab Chip*, 2008, **8**, 198–220.
35. Martin, K., Henkel, T., Baier, V., Grodrian, A., Schon, T., Roth, M., Kohler, J. M. and Metze, J. Generation of large numbers of separated microbial populations by cultivation in segmented-flow microdevices. *Lab Chip*, 2003, **3**, 202–207.
36. Gastrock, G., Lemke, K., Römer, R., Howitz, S., Bertram, J., Hottenrott, M. and Metze, J. Protein-processing platform (3P) – A new concept for the characterization of cell cultures in the mL-scale using microfluidic components. *Eng. Life Sci.*, 2008, **8**, 73–80.
37. Tchavtchavadze, M. B., Perrier, M. and Jolicoeur, M. Small scale bioreactor platform for bioprocess optimisation. *Pharmaceut. Eng.*, 2007, **27**, 1–10.
38. Simon, J., Saffer, S. and Kim, C.-J. A liquid-filled microrelay with a moving mercury microdrop. *J. Microelectromech. Syst.* 1997, **6**, 208–216.
39. Hayes, R. A. and Feenstra, B. J. Video-speed electric paper based on electrowetting. *Nature*, 2003, **425**, 383–385.
40. Kuiper, S. and Hendriks, B. H. W. Variable-focus liquid lens for miniature cameras. *Appl. Phys. Lett.*, 2004, **85**, 1128–1130.
41. Takagi, J., Yamada, M., Yasuda, M. and Seki, M. Continuous particle separation in a micro-channel having asymmetrically arranged multiple branches. *Lab Chip*, 2005, **5**, 778–784.
42. Yamada, M. and Seki, M. Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics. *Lab Chip*, 2005, **5**, 1233–1239.
43. Jäggi, R. D., Sandoz, R. and Effenhauser, C. S. Microfluidic depletion of red blood cells from whole blood in high-aspect-ratio microchannels. *Microfluid. Nanofluid.*, 2007, **3**, 47–53.
44. Choi, S. and Park, J. K. Continuous hydrophoretic separation and sizing of microparticles using slanted obstacles in a microchannel. *Lab Chip*, 2007, **7**, 890–897.
45. Zhang, C. X. and Manz, A. High-speed free-flow electrophoresis on chip. *Anal. Chem.*, 2003, **75**, 5759–5766.
46. Jones, T. B., Fowler, J. D., Chang, Y. S and Kim, C. J. Frequency-based relationship of electrowetting and dielectrophoretic liquid microactuation. *Langmuir*, 2003, **19**, 7646–7651.
47. Jones, T. B. On the relationship of dielectrophoresis and electrowetting. *Langmuir*, 2002, **18**, 4437–4443.
48. Pollack, M. G., Fair, R. B. and Shenderov, A. D. Electrowetting-based actuation of liquid droplets for microfluidic applications. *Appl. Phys. Lett.*, 2000, **77**, 1725–1726.
49. Pollack, M. G., Shenderov, A. D. and Fair, R. B. Electrowetting-based actuation of droplets for integrated microfluidics. *Lab Chip*, 2002, **2**, 96–101.
50. Washizu, M. Electrostatic actuation of liquid droplets for micro-reactor applications. *IEEE Trans. Ind. Appl.*, 1998, **34**, 732–737.
51. Moon, H., Cho, S. K., Garell, R. L. and Kim, C. J. Low voltage electrowetting-on-dielectric. *J. Appl. Phys.*, 2002, **92**, 4080–4087.

52. Pamme, N. and Wilhelm, C. Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis. *Lab Chip*, 2006, **6**, 974–980.
53. Laser, D. J. and Santiago, J. G. A review of micropumps. *J. Micromech. Microeng.*, 2004, **14**, R35–R64.
54. Gascoyne, P. R. and Vykoukal, J. Particle separation by dielectrophoresis. *Electrophoresis*, 2002, **23**, 1973–1983.
55. van Lintel, H. T. G., van de Pol, F. C. M. and Bouwstra, S. A piezoelectric micropump based on micromachining of silicon. *Sensors Actuators*, 1988, **15**, 153–167.
56. Nisar, A., Afzulpurkar, N., Mahaisavariya, B. and Tuantranont, A. MEMS-based micropumps in drug delivery and biomedical applications. *Sensors Actuators B*, 2008, **130**, 917–942.
57. Zhong, J., Yi, M. and Bau, H. H. Magneto hydrodynamic (MHD) pump fabricated with ceramic tapes. *Sensors Actuators A: Physical*, 2002, **96**, 59–66.
58. Xu, D., Wang, L., Ding, G., Zhou, Y., Yu, A. and Cai, B. Characteristics and fabrication of NiTi/Si diaphragm micropump. *Sensors Actuators A: Physical*, 2001, **93**, 87–92.
59. Yokoyama, Y., Takeda, M., Umemoto, T. and Ogushi, T. Thermal micro pumps for a loop-type micro channel. *Sensors Actuators A: Physical*. 2004, **111**, 123–128.
60. Salimi-Moosavi, H., Tang, T. and Harrison, D. Electroosmotic pumping of organic solvents and reagents in microfabricated reactor chips. *J. Am. Chem. Soc.*, 1997, **119**, 8716–8717.
61. Li, P. C. and Harrison, D. J. Transport, manipulation and reaction of biological cells on-chip using electrokinetic effects. *Anal. Chem.*, 1997, **69**, 1564–1568.
62. Manz, A., Fetting, J. C., Verpoorte, E., Lude, H., Widmer, H. M. and Harrison, D. J. Micro-machining of monocrystalline silicon and glass for chemical analysis systems. A look into next century's technology or just a fashionable craze? *Trends Anal. Chem.*, 1991, **10**, 144–149.
63. Terry, S. C., Jerman, J. H. and Angell, J. B. A gas chromatographic air analyzer fabricated on a silicon wafer. *IEEE Trans. Electron. Devices*, 1979, **ED-26**, 1880–1886.
64. Oh, H. J., Kim, S. H., Baek, J. Y., Seong, G. H. and Lee, S. H. Hydrodynamic micro-encapsulation of aqueous fluids and cells via 'on the fly' photopolymerization. *J. Micromech. Microeng.*, 2006, **16**, 285–291.
65. Choi, C. H., Jung, J. H., Rhee, Y., Kim, D. P., Shim, S. E. and Lee, C. S. Generation of monodisperse alginate microbeads and in situ encapsulation of cell in microfluidic device. *Biomed. Microdevices*, 2007, **9**, 855–862.
66. Tan, Y. C., Hettiarachchi, K., Siu, M., Pan, Y. P. and Lee, A. P. Controlled microfluidic encapsulation of cells, proteins, and microbeads in lipid vesicles. *J. Am. Chem. Soc.*, 2006, **128**, 5656–5658.
67. Yamada, M., Nakashima, M. and Seki, M. Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel. *Anal. Chem.*, 2004, **76**, 5465–5471.
68. Shevkoplyas, S. S., Yoshida, T., Munn, L. L. and Bitensky, M. W. Biomimetic design of a microfluidic device for auto-separation of leukocytes from whole blood. *Anal. Chem.*, 2005, **77**, 933.
69. Gomez-Sjoberg, R., Leyrat, A. A., Pirone, D. M., Chen, C. S. and Quake, S. R. Versatile, fully automated, microfluidic cell culture system. *Anal. Chem.*, 2007, **79**, 8557–8563.
70. Nevill, J. T., Cooper, R., Dueck, M., Breslauer, D. N. and Lee, L. P. Integrated microfluidic cell culture and lysis on a chip. *Lab Chip*, 2007, **7**, 1689–1695.
71. Melin, J. and Quake, S. R. Microfluidic large-scale integration: The evolution of design rules for biological automation. *Ann. Rev. Biophys. Biomolecular Structure*, 2007, **36**, 213–231.
72. Talary, M. S., Burt, J. P. H. and Pethig, R. Future trends in diagnosis using laboratory-on-a-chip technologies. *Parasitology*, 1998, **117**, S191–S203.
73. Kopp, M. U., De Mello, A. and Manz, J. A. Chemical amplification: Continuous-flow PCR on a chip. *Science*, 1998, **280**, 1046–1048.
74. Wildinga, P., Kricka, L. J., Cheng, J., Hvizhia, G., Shoffner, M. A. and Fortina, P. Integrated cell isolation and polymerase chain reaction analysis using silicon microfilter chambers. *Analyt. Biochem.*, 1998, **257**, 95–100.

75. Shoffner, M. A., Cheng, J., Hvichia, G. E., Kricka, L. J. and Wilding, P. Chip PCR. I: Surface passivation of microfabricated silicon-glass chips for PCR. *Nucleic Acids Res.*, 1996, **24**, 375–379.
76. Cheng, J., Shoffner, M. A., Hvichia, G. E., Kricka, L. J. and Wilding, P. Chip PCR. II: Investigation of different PCR amplification systems in microfabricated silicon-glass chips. *Nucleic Acids Res.*, 1996, **24**, 380–385.
77. Auroux, P. A., Koc, Y., DeMello, A., Manz, A. and Day, P. J. R. Miniaturised nucleic acid analysis. *Lab Chip*, 2004, **4**, 534–546.
78. Wang, W., Li, Z. X., Luo, R., Lu, S. H., Xu, A. D. and Yang, Y. J. Droplet-based micro oscillating-flow PCR chip. *J. Micromech. Microeng.*, 2005, **15**, 1369–1377.
79. Beer, N. R., Hindson, B. J., Wheeler, E. K., Hall, S. B., Rose, K. A., Kennedy, I. M. and Colston, B. W. On-chip, real-time, single-copy polymerase chain reaction in picoliter droplets. *Anal. Chem.*, 2007, **79**, 8471–8475.
80. Liu, R. H., Yang, J. N., Lenigk, R., Bonanno, J. and Grodzinski, P. Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection. *Analyt. Chem.*, 2004, **76**, 1824–1831.
81. Urban, G. A. Micro- and nanobiosensors – State of the art and trends. *Meas. Sci. Technol.*, 2009, **20**, 012001.
82. Chmela, E. and Tjissen, R. A chip system for size separation of macromolecules and particles by hydrodynamic chromatography. *Analyt. Chem.*, 2002, **74**, 3470–3475.
83. Blom, M. T., Chmela, E., Gardeniers, J. G. E., Tjissen, R., Elwenspoek, M. and van den Berg, A. Design and fabrication of a hydrodynamic chromatography chip. *Sensors Actuators B: Chemical*, 2002, **82**, 111–116.
84. Niemeyer, C. M. and Blohm, D. DNA microarray. *Angew. Chemie Int. Ed.*, 1999, **38**, 2865–2869.
85. Blohm, D. H. and Guiseppi-Elie, A. New developments in microarray technology. *Curr. Opin. Biotechnol.*, 2001, **12**, 41–47.
86. Pirrung, M. C. How to make a DNA chip. *Angew. Chemie Int. Ed.*, 2002, **41**, 1276–1289.
87. Jung, A. DNA chip technology. *Anal. Bioanal. Chem.*, 2002, **372**, 41–42.
88. Saliterman, S. S. Education, bioMEMS and the medical microdevice revolution. *Expert. Rev. Med. Devices*, 2005, **2**, 515–519.
89. Fan, Z. H. and Ricco, A. J. Plastic microfluidic devices for DNA and protein analyses. In *BioMEMS and Biomedical Nanotechnology: Micro/Nano Technology for Genomics and Proteomics* (Ozkan, M., Heller, M. J. and Ferrari, M., eds). Vol. II, Springer, 2006, 311–328.
90. Wagenknecht, H. A. Photoinduced electron transport in DNA. In *Nanobiotechnology: Bio-inspired Devices and Materials of the Future* (Shoseyov, O. and Levy, I., eds). Hamana Press, Totowa, New Jersey, 2008, 89–106.
91. Drummond, T. G., Hill, M. G. and Barton, J. K. Electrochemical DNA sensors. *Nat. Biotechnol.*, 2003, **21**, 1192–1199.
92. Porath, D., Cuniberti, G. and Di Felice, R. Charge transport in DNA-based devices. *Top. Curr. Chem.*, 2004, **237**, 183–227.
93. Köster, S., Angile, F. E., Duan, H., Agresti, J. J., Wintner, A., Schmitz, C., Rowat, A. C., Merten, C. A., Pisignano, D., Griffiths, A. D. and Weitz, D. A. Drop-based microfluidic devices for encapsulation of single cells. *Lab Chip*, 2008, **8**, 1110–1115.
94. Sun, T., Holmes, D., Gawad, S., Green, N. G. and Morgan, H. High speed multi-frequency impedance analysis of single particles in a microfluidic cytometer using maximum length sequences. *Lab Chip*, 2007, **7**, 1034–1040.
95. Gawad, S., Sun, T., Green, N. G. and Morgan, H. Impedance spectroscopy using maximum length sequences: Application to single cell analysis. *Rev. Sci. Instruments*, 2007, **78**, 054301-1.
96. Morgan, H., Sun, T., Holmes, D., Gawad, S. and Green, N. G. Single cell dielectric spectroscopy. *J. Phys. D: Appl. Phys.*, 2007, **40**, 61–70.

97. Fu, A. Y., Spence, C., Scherer, A., Arnold, F. H. and Quake, S. R. A microfabricated fluorescence-activated cell sorter. *Nature Biotechnol.*, 1999, **17**, 1109–1111.
98. Zemmann, A. J., Schnell, E., Volgger, D. and Bonn, G. K. Contactless conductivity detection for capillary electrophoresis. *Analyt. Chem.*, 1998, **70**, 563–567.
99. Pumera, M., Wang, J., Opekar, F., Jelínek, I., Feldman, J., Löwe, H. and Hardt, S. Contactless conductivity detector for microchip capillary electrophoresis. *Analyt. Chem.*, 2002, **74**, 1968–1971.
100. Lichtenberg, J., de Rooij, N. F. and Verpoorte, E. A microchip electrophoresis system with integrated in-plane electrodes for contactless conductivity detection. *Electrophoresis*, 2002, **23**, 3769–3780.
101. Lee, C.-Y., Chen, C. M., Chang, G.-L., Lin, C.-H. and Fu, L.-M. Fabrication and characterization of semicircular detection electrodes for contactless conductivity detector – CE microchips. *Electrophoresis*, 2006, **27**, 5043–5050.
102. Gorbatsova, J., Jaanus, M. and Kaljurand, M. Digital microfluidic sampler for a portable capillary electropherograph. *Analyt. Chem.*, 2009, **81**, 8590–8595.
103. da Silva, J. A. F. and do Lago, C. L. An oscillometric detector for capillary electrophoresis. *Analyt. Chem.*, 1998, **70**, 4339–4343.
104. Abad-Villar, E. M., Kubáň, P. and Hauser, P. C. Determination of biochemical species on electrophoresis chips with an external contactless conductivity detector. *Electrophoresis*, 2005, **26**, 3609–3614.
105. Cabrera, C. R. and Yager, P. Continuous concentration of bacteria in a microfluidic flow cell using electrokinetic techniques. *Electrophoresis*, 2001, **22**, 355–362.
106. Lu, H., Schmidt, M. A. and Jensen, K. F. A microfluidic electroporation device for cell lysis. *Lab Chip*, 2005, **5**, 23–29.
107. Li, Y. L., Dalton, C., Crabtree, H. J., Nilsson, G. and Kaler, K. Continuous dielectrophoretic cell separation microfluidic device. *Lab Chip*, 2007, **7**, 239–248.
108. Doh, I. and Cho, Y. H. A continuous cell separation chip using hydrodynamic dielectrophoresis (DEP) process. *Sens. Actuators A: Physical*, 2005, **121**, 59–65.
109. Huang, Y. and Pethig, R. Electrode design for negative dielectrophoresis. *Meas. Sci. Technol.*, 1991, **2**, 1142–1146.
110. Gascoyne, P. R. C., Wang, X.-B., Huang, Y. and Becker, F. F. Dielectrophoretic separation of cancer cells from blood. *IEEE Trans. Industry Applications*, 1997, **33**, 670–678.
111. Wang, X.-B., Huang, Y., Wang, X., Becker, F. F. and Gascoyne, P. R. C. Dielectrophoretic manipulation of cells with spiral electrodes. *Biophys. J.*, 1997, **72**, 1887–1899.
112. Broche, L. M., Bhadal, N., Lewis, M. P., Porter, S., Hughes, M. P. and Labeed, F. H. Early detection of oral cancer – Is dielectrophoresis the answer? *Oral Oncology*, 2007, **43**, 199–203.
113. Cen, E. G., Dalton, C., Li, Y., Adamia, S., Pilarski, L. M. and Kaler, K. V. I. S. A combined dielectrophoresis, traveling wave dielectrophoresis and electrorotation microchip for the manipulation and characterization of human malignant cells. *J. Microbiol. Methods*, 2004, **58**, 387–401.
114. Jones, T. B. *Electromechanics of Particles*. Cambridge University Press, 1995.
115. Kirby, B. J. *Micro- and Nanoscale Fluid Mechanics: Transport in Microfluidic Devices*. Cambridge University Press, 2010.
116. Cahill, B. P., Heyderman, L. J., Gobrecht, J. and Stemmer, A. Electro-osmotic streaming on application of traveling-wave electric fields. *Phys. Rev.*, 2004, **70**, 036305.
117. Kirby, B. J., Wheeler, A. R., Zare, R. N., Fruetela, J. A. and Shepodd, T. J. Programmable modification of cell adhesion and zeta potential in silica microchips. *Lab Chip*, 2003, **3**, 5–10.
118. Chun, M.-S., Shim, M. S. and Choi, N. W. Fabrication and validation of a multi-channel type microfluidic chip for electrokinetic streaming potential devices. *Lab Chip*, 2006, **6**, 302–309.
119. Estes, M. D., Do, J. and Ahn, C. H. On chip cell separator using magnetic bead-based enrichment and depletion of various surface markers. *Biomed. Devices*, 2009, **11**, 509–515.

120. Gijs, M. Magnetic bead handling on-chip: New opportunities for analytical applications. *Microfluid. Nanofluid.*, 2004, **1**, 22–40.
121. Ogiue-Ikeda, M., Sato, Y. and Ueno, S. A new method to destruct targeted cells using magnetizable beads and pulsed magnetic force. *IEEE Trans. Nanobiosci.*, 2003, **2**, 262–265.
122. Valberg, P. A. and Butler, J. P. Magnetic particle motions within living cells – Physical theory and techniques. *Biophys. J.*, 1987, **52**, 537–550.
123. Wang, N., Butler, J. P. and Ingber, D. E. Mechanotransduction across the cell surface and through the cytoskeleton. *Science*, 1993, **260**, 1124–1127.
124. Egatz-Gómez, A., Melle, S., García, A. A., Lindsay, S. A., Márquez, M., Picraux, S. T., Taraci, J. L., Clement, T., Yang, D., Hayes, M. A. and Gust, D. Discrete magnetic microfluidics, *Appl. Phys. Lett.*, 2006, **89**, 034106.
125. Lehmann, U., Hadjidj, S., Parashar, V. K., Vandevyver, C., Rida, A. and Gijs, M. A. M. Two-dimensional magnetic manipulation of microdroplets on a chip as a platform for bioanalytical applications. *Sensors Actuators B: Chemical*, 2006, **117**, 457–463.
126. Xia, Y. and Whitesides, G. M. Soft lithography. *Ann. Rev. Mater. Sci.*, 1998, **28**, 153–184.
127. Kumar, A. and Whitesides, G. M. Features of gold having micrometer to centimeter dimensions can be formed through a combination of stamping with an elastomeric stamp and an alkanethiol ‘ink’ followed by chemical etching. *Appl. Phys. Lett.*, 1993, **63**, 2002–2004.
128. Harrison, D. J., Glavina, P. G. and Manz, A. Towards miniaturized electrophoresis and chemical analysis systems on silicon: An alternative to chemical sensors. *Sensors Actuators B: Chemical*, 1993, **10**, 107–116.
129. Wilding, P., Shoffner, M. A. and Kricka, L. PCR in a silicon microstructure. *J. Clin. Chem.*, 1994, **40**, 1815–1818.
130. Raymond, D. E., Manz, A. and Widmer, H. M. Continuous separation of high molecular weight compounds using a microliter volume free-flow electrophoresis microstructure. *Anal. Chem.*, 1996, **68**, 2515–2522.
131. Fan, Z. H. and Harrison, D. J. Micromachining of capillary electrophoresis injectors and separators of glass chips and evaluation of flow at capillary intersections. *Analyt. Chem.*, 1994, **66**, 177–184.
132. Jacobson, S. C., Hergenroder, R., Koutny, L. B., Warmack, R. J. and Ramsey, J. M. Effects of injection schemes and column geometry on the performance of microchip electrophoresis devices. *Analyt. Chem.*, 1994, **66**, 1107–1113.
133. Roberts, M. A., Rossier, J. S., Bercier, P. and Girault, H. H. UV laser machined polymer substrates for the development of microdiagnostic systems. *Analyt. Chem.*, 1997, **69**, 2035–2042.
134. Soper, S. A., Ford, S. M., Qi, S., McCarley, R. L., Kelly, K. and Murphy, M. C. Peer reviewed: Polymeric microelectromechanical systems. *Analyt. Chem.*, 2000, **72**, 643A–651A.
135. Friend, J. and Yeo, L. Fabrication of microfluidic devices using polydimethylsiloxane. *Bio-microfluidics*, 2010, **4**, 026502.
136. Toepke, M. W. and Beebe, D. J. PDMS absorption of small molecules and consequences in microfluidic applications. *Lab Chip*, 2006, **6**, 1484–1486.
137. Satas, D. *Coatings Technology Handbook*. Marcel Dekker Inc., 1991.
138. Christensen, C., de Reus, R. and Bouwstra, S. Tantalum oxide thin films as protective coatings for sensors. *J. Micromech. Microeng.*, 1999, **9**, 113–118.
139. Joshi, P. C. and Cole, M. W. Influence of postdeposition annealing on the enhanced structural and electrical properties of amorphous and crystalline Ta₂O₅ thin films for dynamic random access memory applications. *J. Appl. Phys.*, 1999, **86**, 15.
140. Cahill, B. P., Giannitsis, A. T., Land, R., Gastrock, G., Pliquett, U., Frense, D., Min, M. and Beckmann, D. Reversible electrowetting on silanized silicon nitride. *Sensors Actuators B*, 2010, **144**, 380–386.

141. Li, J., Fu, J., Cong, Y., Wu, Y., Xue, L. and Han, Y. Macroporous fluoropolymeric films templated by silica colloidal assembly: A possible route to super-hydrophobic surfaces. *Appl. Surface Sci.*, 2006, **252**, 2229–2234.
142. Hoque, E., DeRose, J. A., Hoffmann, P., Bhushan, B. and Mathieu, H. J. Alkylperfluorosilane self-assembled monolayers on aluminum: A comparison with alkylphosphonate self-assembled monolayers. *J. Phys. Chem. C*, 2007, **111**, 3956–3962.
143. Hoque, E., DeRose, J. A., Hoffmann, P. and Mathieu, H. J. Robust perfluorosilanized copper surfaces. *Surf. Interface Anal.*, 2006, **38**, 62–68.
144. Bayiati, P., Tserepi, A., Petrou, P. S., Kakabakos, S. E., Misiakos, K. and Gogolides, E. Electrowetting on plasma-deposited fluorocarbon hydrophobic films for biofluid transport in microfluidics. *J. Appl. Phys.*, 2007, **101**, 103306.
145. Bayiati, P., Tserepi, A., Petrou, P. S., Misiakos, K., Kakabakos, S. E., Gogolides, E. and Cardinaud, C. Biofluid transport on hydrophobic plasma-deposited fluorocarbon films. *Microelectronic Eng.*, 2007, **84**, 1677–1680.
146. Seyrat, E. and Hayes, R. A. Amorphous fluoropolymers as insulators for reversible low-voltage electrowetting. *J. Appl. Phys.*, 2001, **90**, 1383–1386.
147. Kedzierski, J. and Berry, S. Engineering the electrocapillary behavior of electrolyte droplets on thin fluoropolymer films. *Langmuir*, 2006, **22**, 5690–5696.
148. Schreiber, F. Structure and growth of self-assembling monolayers. *Progress in Surface Science*, 2000, **65**, 151–256.
149. Carlen, E. T., Heng, K. H., Bakshi, S., Pareek, A. and Mastrangelo, C. H. High-aspect ratio vertical comb-drive actuator with small self-aligned finger gaps. *J. Microelectromech.*, 2005, **14**, 1144–1154.
150. Christensen, T. B., Pedersen, C. M., Grondahl, K. G., Jensen, T. G., Sekulovic, A., Bang, D. D. and Wolff, A. J. PCR biocompatibility of lab-on-a-chip and MEMS materials. *J. Micromech. Microeng.*, 2007, **17**, 1527–1532.
151. Schumacher, J. T., Grodrian, A., Kremin, C., Hoffmann, M. and Metze, J. Hydrophobic coating of microfluidic chips structured by SU-8 polymer for segmented flow operation. *J. Micromech. Microeng.*, 2008, **18**, 055019.
152. Kotzar, G., Freas, M., Abel, P., Fleischman, A., Roy, S., Zorman, C., Moran, J. M. and Melzak, J. Evaluation of MEMS materials of construction for implantable medical devices. *Biomaterials*, 2002, **23**, 2737–2750.
153. Voskerician, G., Shive, M. S., Shawgo, R. S., von Recum, H., Anderson, J. M., Cima, M. J. and Langer, R. Biocompatibility and biofouling of MEMS drug delivery device. *Biomaterials*, 2003, **24**, 1959–1967.
154. Hernandez, P. R., Taboada, C., Leija, L., Tsutsumi, V., Vazquez, B., Valdes-Perezgasga, F. and Reyes, J. L. Evaluation of biocompatibility of pH-ISFET materials during long-term subcutaneous implantation. *Sensors Actuators B*, 1998, **46**, 133–138.
155. Jeong, J. H., Moon, Y. M., Kim, S. O., Yun, S. S. and Shin, H. I. Human cartilage tissue engineering with pluronic and cultured chondocyte sheet. *Key Eng. Mater.*, 2007, **342–343**, 89–92.
156. Klapperich, C. M. Microfluidic diagnostics: Time for industry standards. *Expert Rev. Medical Devices*, 2009, **6**, 211–213.
157. Pfohl, T., Mugele, F., Seemann, R. and Herminghaus, S. Trends in microfluidics with complex fluids. *Chemphyschem*, 2003, **4**, 1291–1298.
158. Thies, W., Urbanski, J. P., Thorsen, T. and Amarasinghe, S. Abstraction layers for scalable microfluidic biocomputers. In *Proc. International Meeting on DNA Computing* (Mao, C. and Yokomori, T., eds). Seoul, Korea, 2006, 308–323.
159. Cox, J. C. and Ellington, A. D. DNA computation function. *Curr. Biol.*, 2001, **11**, R336.
160. Yurke, B., Mills, A. P. Jr and Cheng, S. L. DNA implementation of addition in which the input strands are separate from the operator strands. *BioSystems*, 1999, **52**, 165–174.
161. Hug, H. and Schuler, R. Strategies for the development of a peptide computer. *Bioinformatics*, 2001, **17**, 364–368.

162. Sakamoto, K., Gouzu, H., Komiya, K., Kiga, D., Yokoyama, S., Yokomori, T. and Hagiya, M. Molecular computation by DNA hairpin formation. *Science*, 2000, **288**, 1223–1226.
163. Boulart, C., Mowlem, M. C., Connelly, D. P., Dutasta, J.-P. and German, C. R. A novel, low-cost, high performance dissolved methane sensor for aqueous environments. *Optics Express*, 2008, **16**, 12607–12617.
164. Sosna, M., Denuault, G., Pascal, R. W., Prien, R. D. and Mowlem, M. Development of a reliable microelectrode dissolved oxygen sensor. *Sensors Actuators B*, 2007, **123**, 344–351.
165. Benazzi, G., Holmes, D., Sun, T., Mowlem, M. C. and Morgan, H. Discrimination and analysis of phytoplankton using a microfluidic cytometer. *IET Nanobiotechnology*, 2007, **1**, 94–101.
166. Patey, M. D., Rijkenberg, M. J. A., Statham, P. J., Mowlem, M., Stinchcombe, M. C. and Achterberg, E. P. Determination of nitrate and phosphate in seawater at nanomolar concentrations. *Trends Analyt. Chem.*, 2008, **27**, 169–182.
167. Burns, M. A., Johnson, B. N., Brahmasandra, S. N., Handique, K., Webster, J. R., Krishnan, M. T., Sammarco, S., Man, P. M., Jones, D., Heldsinger, D., Mastrangelo, C. H. and Burke, D. T. An integrated nanoliter DNA analysis device. *Science*, 1998, **282**, 484–487.

Biomeditsiiniliste kiiplaborite valmistamine

Athanasios T. Giannitsis

Kiiplaborid on miniatuursete analüütiliste seadmete klassi kuuluvad seadmed, mis ühendavad endas voolise, elektroonika ja sensorika ning on ette nähtud biokeemiliste vedelike analüüsimiseks. Täiendavate abivahenditena kergendavad kiiplaborid vedelike transporti, sorteerimist, segustamist või lahutamist. Nende miniatuursete seadmete tähtsus ilmneb laboratoorsete protseduuride automatiseerimises, mis vähendab tunduvalt biomeditsiiniliste katsete ja laboratoorsete toimingute aega. Antud ülevaateartiklis on kirjeldatud arvukaid kiiplaborite valmistamise meetodeid ja protseduure, samuti on vaadeldud võimalikke tulevikuarenguid.