Fabrication methods for microfluidic lab-on-chips

A. T. Giannitsis[1*], M. Min[1]

[1]*Department of Electronics, Tallinn Technical University, Ehitajate tee 5, 19086 Tallinn, Estonia*
[*]*Tel: +372 6202164, Fax: +372 6202151, E-mail: thanasis@elin.ttu.ee*

ABSTRACT: Microfluidic lab-on-chips are miniaturized biosensors that integrate fluidic, electronics, thermal elements or optical apparatuses for analysing electrolytic or biochemical samples. Supplementary to their analysing capabilities, the microfluidic lab-on-chips can process, control, separate or sort fluidic samples by means of hydrodynamic manipulation, thermal gradients, electrical or optical actuation. The significance of the lab-on-chips lies on the potential of automating laboratory procedures which highly reduce the time of the laboratory tests. We review numerous fabrication methods and procedures that we have experienced by designing such devices.

1 Introduction

Lab-on-chips is a technology concept for producing miniaturized fluidic biochips. Lab-on-chip technology emphasises integration, chip programmability, increased sensitivity, minimal reagent consumption, sterilisation, and high-throughput sample detection and separation schemes. Several lab-on-chip clinical assessments include cell analysis, cytometry, polymerase chain reaction, blood preparation and bioassays. Technical limitations such as size, liquid handling, power consumption, but also chip stability and biocompatibility shall all be accounted in the design of a biochip.

A typical lab-on-chip contains microchannels which allow liquid samples to flow within them, but also contains measuring, sensing and controlling components such as microvalves, fluidic mixers, microelectrodes, thermal elements, or optical apparatuses. If optical sensors are used on a biochip, its microchannels should be transparent and should not disrupt the light beam. Transparent electrodes might be required in the case that the biochip combines microelectrodes and optical mechanisms together.

The lab-on-chip fabrication process is very comparable to microelectronics fabrication procedures. The process requires microfabriacation and clean room facility. The process includes: (a) metallisation of a pair of silicon or glass substrates or a pair of polycarbonate substrates, which configures the bottom and the cover of the fluidic chip; (b) fabrication of chromium photomasks which define the patterns of microelectrodes and microchannels on the substrates; (c) micromachining to shape structures, or apply photolithography which involves spinning-off photoresist layers, exposing to ultraviolet radiation and etch to reveal structures; (d) dish the substrates with diamond saw or ultrasound and alike produce holes for the inlets, or in the case of polycarbonate substrate mill and drill mechanically; (e) bonding the top and bottom plates by means of anodic or thermal bonding in order to crosslink the opposite structures; (f) fit the chip into a customised holder to join the fluidic connections and electrical contacts; (g) electronics, such as a microprocessor, can be embedded on the chip by fitting the electronics onto a circuit board attached on the holder.

2 Fabrication materials

Lab-on-chips can be fabricated by a number of materials and manufacturing techniques. These techniques are analogous to the microelectronic fabrication methods. Materials can be (a) silicon, glass or polymers for the microfluidic parts, (b) metals like gold, platinum, or titanium for the conductive parts, and (c) electroactive polymers or other elastomers for the moving parts.

Silicon (Si) and glass (SiO_2) wafers are commonly used as substrates for making most lab-on-chips used today. Precision, miniaturisation, cost effectiveness, large-scale production and ability to incorporate electronics, makes silicon attractive for manufacturing lab-on-chip devices. Silicon and glass are very reliable as in the form of a wafer substrate demonstrate strong crystalline structure and can survive reusage. The standard procedure for producing fluidic chips from silicon or glass involves: (a) deposition of material layers on silicon or glass wafer; (b) patterning these layers with photolithography to formulate shapes of microchannels and microelectrodes; (c) etch the patterns chemically in order to produce the desired structures.

Polymers, like polycarbonates, have varied interfacial and structural characteristics. Polymers have disadvantageous optical properties compared to glass, like lower light transmittance or higher self-fluorescence emission, but better thermal properties [1]. Microfluidic channels can be made from polymers by plastic molding or micromachining. Particularly fluoropolymers, such as polytetrafluoroethylene (PTFE $(C_2F_4)_n$) are handy substrates for structuring microchannels as they are soft for milling them and also hydrophobic, and their hydrophobicity highly eases liquids to flow within the

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microchannels. For rapid prototyping, casting polydimethylsiloxane (PDMS $(C_2H_6OSi)_n$) by means of silicon or smoothed metallic masters can produce PDMS chips. PDMS provides flexibility, hydrophobicity and seals the chips tightly upon application of uniform mechanical pressure. PDMS can irreversibly seals various substrates, such as glass, Si, Si_3N_4, polyethylene, glassy carbon, oxidised polystyrene, fluorocarbons and metal [1]. Soft lithography is a fast method for rapid prototyping and PDMS is employed in soft lithography as mask for patterning microchannels on glass substrates. Compared to glass, PDMS has lower thermal conductivity much higher hydrophobicity, and both of these properties qualify PDMS as suitable for making fluidic microchannels. On the other hand PDMS is swollen to many organic solvents like oils, but unaffected by water, nitromethane, ethylene glycol, acetonitrile, perfluorotributylamine, perfluorodecalin and propylene carbonate. The compatibility of PDMS to other organic solvents can be improved by coating the microchannel with sodium silicate [1]. Yet, due to pliability of PDMS electrodes cannot be developed on it.

Metal films like gold (Au), platinum (Pt) and titanium (Ti), or metalloid films like Indium Tin Oxide (ITO), are suitable for patterning microelectrodes for biochips. Gold and platinum are highly biocompatible, chemically inert and thus are considered suitable metals for contacting biological solutions. Gold, platinum, titanium or ITO can be sputtered or deposited by evaporation on substrates. The electrode patterns can be shaped by chemical etching or lift-off process, which obtains resolution of few micrometers. The chemical etching method is described below. Alternatively, it is possible to produce patterns by milling the metal film with resolution of few tens to hundreds micrometers. Electrodes in biochips are preferred coated for preventing electrolysis of the contacting fluid. But in the case of low voltage applications, such as bioimpedance, the electrodes can be bare. Other usage of metal films entails production of masks, overlayers or adhesion layers. Yet, manufacturing heat elements (200nm Pt/20nm Ti) by means of electroplating can be obtained if gold is electrodeposited onto an area of the Pt/Ti layer to form the heater [1]. Pt thin films on glass substrate can withstand thermal bonding and thus can be used for wiring contacts.

For controlling the flow of the liquids, movable microapparatuses might be integrated in biochips. The electroactive polymers are an important class of materials for fabricating microvalves or micropumps, as they can be precisely controlled electrically.

3 Coating materials

The metallization of silicon wafers or polymer substrates is the primary procedure for patterning microelectrodes on them. Gold and platinum are widely preferable for patterning microelectrodes, due to their (a) excellent conductivity that makes them ideal for transmitting electric signals undistorted, and (b) biocompatibility. Both gold and platinum can be sputtered or chemically deposited on silicon or polymer substrates.

The density of metal films that are made of chemical deposition becomes bit lower than of the bulk metals. The produced metal film might contain defects such as pores and inclusions of foreign matter of 20-300Å in diameter. Further, the mechanical strength of the metal films depends on the solution composition and its deposition rate. For instance tensile strengths of 40-50 kg/mm^2 might be obtained at 50-70^{o}C [2]. The electrical conductivity of chemically deposited metal films is usually lower than of pure metals. The optical properties however are less varied and do not differ much of those of pure metals. The optical property of a fabrication material should be accounted for lab-on-chips that work along with optical sensing apparatuses.

Yet, it is possible to develop gold electrode patterns of micrometer thickness on polymer substrates by means of aerosol spraying metallization, where gold with amines are employed together with hydrazine as reducer, which results to gold films of thickness of 400 nm/min [2]. Similarly, platinum can be deposited with adjustable process rate of 500-2000 nm/h using borohydride or hydrazine as reducing agents.

Insulating films of nanometer thickness are useable in insulating electrically fluids from electrodes for preventing hydrolysis. For insulation, it is possible to accurately grow oxide layers on metal electrodes by means of deposition, sputtering, or anodic or thermal oxidation. The oxide films further protect the microelectrodes from chemical aggressions, especially if the microelectrodes come to direct contact with electrolytes, biological solutions, or acids. The oxide protective films prevent electrolysis as they highly resist against currents produced by high voltage electrokinetic phenomena in fluids (electrophoresis, dielectrophoresis, electrowetting). However in low voltage impedimetric applications (range of mV) it is widely preferred to directly contact the measuring liquid samples with bare electrodes. Thermally gown oxide films, like silicon dioxide (SiO_2) and the self-healing tantalum pentoxide (Ta_2O_5) are very appropriate options for robust electric insulations due to their high breakdown resistance [3,4,5]. Thermally grown SiO_2 layers might form thicknesses of only 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 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 to Ta_2O_5. Strontium titanate ($SrTiO_3$) is an oxide of strontium and titanium, it has very large permittivity and thus it is suitable for high voltage electric insulation.

Transparent conductive films, like Indium Tin Oxide (ITO), are favourable electrodes in lab-on-chips because their transparency allows microscopy. Indium Tin Oxide films are mostly deposited on substrates by electron beam evaporation, physical vapor deposition, or sputtering.

Prior to coating, chemical treatment of the interior of silicon or glass microchannels can enhance adhesion of films on the microchannel walls. A glass microchannel can be treated with ethanol, acetone, or a mixture of strong acids. Likewise, in polymers the adhesion of a film develops faster on prepared and cleaned surfaces and this results to lasting coatings. Some molded thermoplastics, processed in high melt temperature followed by rapid cooling, are easier to coated because their surface activity increases film's adhesion. Plasma treatment also cleans organic matter from the interior of a microfluidic chip. Plasma cleaning can either be performed before the chip is assembled or afterwards. In the second case capillary plasma can be produced within the fluidic channels by (a) sealing the channel inlets with needle electrodes, (b) generating vacuum after pumping out the air from the channel, and (c) applying high voltage pulses of few thousands of volts for initiating electric spark which rapidly evolve into a plasma arc which can be visible through a transparent channel. Reaction gasses for producing plasma are neon, helium and argon.

4 Deposition methods

Chemical vapor deposition is a process for producing 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^{o}C and pressures between millitorrs to torrs, with volatile precursors that react and decompose on the substrate a film of 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 gasses like nitrogen (N_2) or argon (Ar), and reactive gasses such as metal halides and hydrocarbons. A typical chemical reaction sequence includes pyrolysis, reduction, oxidation, hydrolysis and coreduction. The volatile byproducts can be blown away from the reaction chamber and neutralised before exposed to the environment. The chemical vapor deposition can also be plasma enhanced, a method that functionalises surfaces and is effective in depositing hydrophobic films on wafers. For example a fluorocarbon hydrophobic film can be formed at an interface by plasma-enhanced chemical vapor deposition of CHF_3. The plasma-enhanced technique can also be used for depositing insulating films such as silicon nitride (Si_3N_4), or plasma coated silicon dioxide (SiO_2). Capillary plasma is a method of producing gas plasma within a microchannel by sealing its inlets with electrode needles, pumping out the air from an outlet, inserting the reaction gasses, and applying high voltage pulses across the needles. The plasma arc that is produced can be clearly visible if the channel is transparent. Capillary plasma deposition of platinum bisacetylacetonate precursor is performed for depositing platinum inside channels. The closely related technique of plasma electrolytic oxidation, or microarc oxidation, can electrochemicaly produce on top of an electrode an oxide coating of micrometer thickness, which provides electric insulation and excellent adhesion.

Adsorption is a chemical deposition method that exploits hydrophilic head groups of self-assembled monolayers of, for example, hydrophobic trichlorosilanes ($Cl_3SiC_nH_{2n+1}$), or thiols (HSC_nH_{2n+1}) that offer spontaneous binding on glass and metal areas [6]. A glass substrate can be functionalized by acidic treatment with 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, and glass is the most obvious surface capable of providing silanol groups.

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

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

5 Etching procedures

In lab-on-chip fabrication, patterning is the transfer of outlines, which define microchannels or electrodes, onto a substrate by means of a photomask. Photolithography produces structures by means of exposing photosensitive resists to ultraviolet light via chromium photomask and then removes the exposed areas in the development process. The exposed regions can then be etched to produce electrodes and fluidic channels. Every wafer substrate undergoes many etching steps before it is completed. We distinguish between two etching processes, the wet and the dry etching:

(a) The wet chemical etching is widely used for producing microelectrodes and microchannels. The wet etching requires acids, bases or mixtures to dissolve metals, silicon and glass, by dipping them into the solution. For example, gold can be etched with iodine solution. Silicon <100> can be etched isotropically with HF-HNO_3, which produces rectangular curved grooves by means of thermally grown SiO_2 mask. KOH etcher can etch anisotropically Si <100> or <110>, where the etching rate depends on the crystallographic orientation, and produces grooves with 55° or 90^{o} walls, respectively. Glass can be etched isotropically using buffered hydrofluoric acid (HF) in HCl or ultrasonic bath that improves smoothness of the etched surface. It is important to mention that the etching rate of any mask should be considerably higher than that of the removable material and for this the etcher should be selected carefully.

(b) The dry etching involves reactive ion etching with plasma, where the substrate is placed inside a chamber where a gas mixture is introduced and becomes ionised by the plasma. The ionised gas mixture reacts with the surface of the material being etched. If the ionised gas is highly energised it removes matter from the substrate. Xenon difluoride (XeF2) is a dry vapor phase isotropic etcher for silicon, originally applied in the technology of lab-on-chips. Its etching selectivity to silicon is very high and does not affects masks made of photoresists, silicon dioxide, silicon nitride or metals.

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 [7,8]. Solidifying one SU8 formation against another by crosslinking the opposing SU8 layers, 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 manufacturing lab-on-chips. Further, the SU8 is suitable of serving as mask on top of silicon wafer in wet etching processes [1].

6 Bonding

After manufacturing the microchannels, the electrodes and the inlets on the base substrate and the cover plate, both plates can bond against each other in order to assemble the chip. It is possible to bond the plates either by (a) anodic bonding, under the requirement of bonding silicon or glass plates (conditions are 200-1100V, 200-450^{o}C); (b) thermal bonding, under the requirement of bonding glass or polymer plates (obtained by spinning-off methylsilses-quioxane or polysiloxane and heating at 150-210^{o}C); or (c) using photopolymerising adhesive for bonding any type of substrate (C_8H_8 benzocyclobutene, or UV-curable resin of μm thickness).

Glass is tricky to bond. To bond two glass substrates the manufacturers prefer anodic bonding where adhesive layer of 200 nm silicon nitride (Si_3N_4) or 120 nm Ni/Cr is used at 1500V, 450^{o}C [1]. If the chip contains only glass structures, annealing at 200 °C for some hours in low vacuum, produces a bond able of withstanding hundreds of N/cm^2. For rapid bonding of glasses, hot press can be used at 570 °C for 10 minutes under 4.7 N/mm^2. After performing anodic or thermal bonding it is not possible to separate the plates anymore. However if polymer adhesive glue is used instead, it is possible by heating to separate the plates apart and rebond the chip.

7 Fluidic and electric connections

Customised chip holder can support tubing connection and wiring the chip. For adhesive-free fluidic connection, a flange-shaped plastic fitting with standard threads can be used along with Teflon tubing. This connector uses metal O-ring that presses the tube's flange against the inlet of the chip and seals tightly. The connector can tightly be screwed into threads existing on the chip holder. Another connection method uses injection-molded connector with internal threads that can withstand pressures of MPa. Adhesive or solder-based chip-to-tube connections can tolerate elevated pressures and solvents.

8 Conclusions

A number of materials, fabrication techniques and assembling methods for manufacturing fluidic lab-on-chips have been overviewed. Lab-on-chips must preferable be made of materials that are biocompatible, chemically inert, reliable and reusable for lifetime. Gold or platinum is intended for structuring conductive elements. Fluoropolymers and silanes are intended 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 sufficient for insulating the conductive parts of the biochips.

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