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Microfluidic devices for biomedical applications

Streszczenie. *W niniejszym artykule przedstawiono zastosowania dwóch układów mikrofluidycznych w diagnostyce biomedycznej. Pierwszy lab-on-a-chip (LOC) przeznaczony jest do elektrochemicznego oznaczania leków psychotropowych w ślinie, natomiast drugi do optycznego wykrywania oraz rozpoznawania bakterii.*

Abstract. *Two types of microfluidic devices demonstrators designed for biomedical applications are presented. The first one is modular lab-on-a-chip (LOC) system, intended for the electrochemical detection of psychotropic drug presence and content in the human saliva. The second device was an optical and microfluidic system for bacteria detection and recognition.* ***(Zastosowanie układów mikrofluidycznych w diagnostyce biomedycznej).***

Słowa kluczowe: lab-on-a-chip, mikrofluidyka, cystometria przepływowa, technologia krzemowa.
Keywords: lab-on-a-chip, microfluidics, flow cytometry, silicon technology.

Introduction

Microfluidic devices have been widely applied for the analytical systems due to their fast response capabilities, low cost and small amounts of necessary, very expensive chemical bioreceptors, such as: antibodies, aptamers or enzymes [1]. Microfluidic systems may be fabricated using: polymers, glass or silicon. Chemical and plasma etching steps, photolithography and mold techniques were applied in technological process sequence to form microchannels, pillars, chambers and other microfluidic elements.

Electrodes for the electrochemical detection may be formed from the deposited metal layers (for instance Au, Pt, Ag, Ti, Cr), metal oxides and chlorides, non-metals (e.g. carbon, diamond like layers) and electroconductive polymers. Computer modeling and simulation were applied as a very useful tool for improvements of the design of device geometry, as well as for the optimization of the technological and functional parameters [2].

Design and technology

Our research was focused on design and manufacturing technology of the lab-on-a-chip (LOC) simple modular system for electrochemical detection (presence and/or content) of psychotropic drugs in the humans' saliva. Such a device may be applied for instance to control car drivers during the routine police control on the road. The test procedure should be fast, simple, non-invasive, and shouldn't require any advanced laboratory support.

Microfluidic chips were formed with use of the SU-8 photoresist layers deposited on the silicon substrates or with use of the silicon moulds shaped by the DRIE process - deep reactive ion etching of silicon (Bosch plasma process). PDMS micro imprints taken from the SU-8 or Si moulds were applied as a microfluidic chips consisting of system of shallow and narrow channels (few tens of micrometers deep and wide), containers, mixers, and other elements.

Microfluidic chips

Moulds applied for the microfluidic PDMS chips formation were fabricated with use of advanced silicon technology. Standard mono-crystalline silicon 4" and 500 µm thick,wafers of arbitrary crystal orientation, resistivity and type of electrical conductivity, were chemically washed and oxidized. To reduce adhesion forces of the PDMS molds to the templates, on each wafer a thin SU-8 photoresist layer was deposited by spin-coating, then exposed (without any masking) to the UV light and crosslinked on the hot plate. To form mold for the microfluidic system, on the top of such a base SU-8 thin layer, the second one - thick SU-8 photoresist layer was deposited. Few tens to few hundred micrometers thick photoresist layer may be applied - thickness of this layer defines depth of the microchannels. The second photoresist layer was also deposited with use of the spin-on technique on each wafer surface and submitted to the photolithography step, this time with use of the glass mask. Glass mask contains shapes of the microchannels defined by the project developed in AutoCAD. Because of the side effects during the long UV exposition of the SU-8 layer, such as thermal influence and surface degradation, the exposition time had to be divided into several phases to enable heat sink to the ambient. Development and photoresist crosslinking on the hot plate were the final technological steps of the template formation, Fig. 1A.

The other method of the templates formation consists of the silicon DRIE etching step. The same type mono-crystalline silicon wafers were applied as the substrates. Standard photolithography step was performed to transfer shapes from the glass mask to the metal (Al) and silicon dioxide (SiO_2) auxiliary layers. Deep silicon plasma etching process consisted of consecutive Si etch and thin polymer layer deposition steps. It results in almost vertical sidewalls of trenches with the aspect ratio up to 1:30 (width-to-depth), Figs. 1B, 1C.

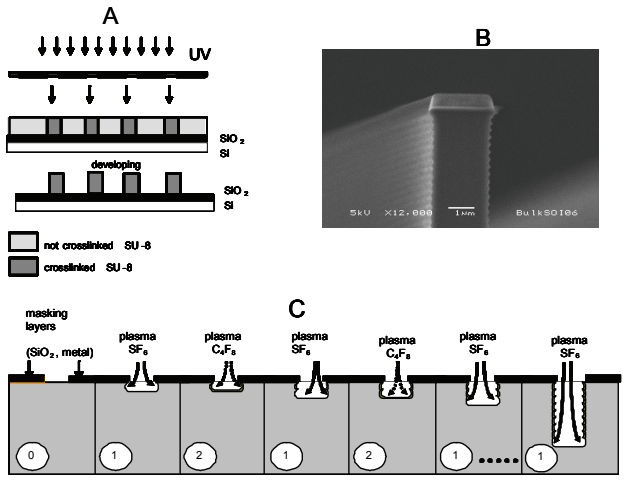

Fig.1. Cross-sectional illustration of the SU-8 templates technology, B. SEM microphotography of the Si vertical wall, formed with application of the DRIE Bosch plasma process, C. schematic illustration of the DRIE Bosch technology.

Microfluidic PDMS molds were formed with use of the SU-8 and silicon templates [3-6, 8-12]. PDMS as a material and its technology are widely known. PDMS molds were trimmed with use of the IR laser – cutting chip edges and holes drilling across the PDMS mould thickness, to enable fluid access to the microchannels. Surface of the PDMS moulds were chemically activated in the oxygen plasma reactor to form PDMS chips in stacks with the microchannels at the interface.

Electrodes.

Silicon chips with metal electrodes were batch-fabricated on the oxidized silicon substrates. 4" silicon wafers and 0.5 mm thick, of arbitrary crystal orientation, arbitrary type of conductivity and arbitrary resistivity were applied. Also "lift-off" technique and Au/Ti or Pt/Ti metal layers with SiO_2 passivation layer on the top were applied. Few nanometer-thick Ti layer was necessary to improve Au or Pt thick layers adhesion to the silicon dioxide layer, Fig. 2. Selected electrodes were electrochemically covered by the thick silver layer and then chlorinated to form reference electrodes. Wafers were diced and then, individual silicon chips were assembled on the PCB sticks and electrically connected to electrical pads on each PCB stick. For mechanical and chemical protection, a very delicate, thin metal wires were manually encapsulated and laminated by the epoxy resin.

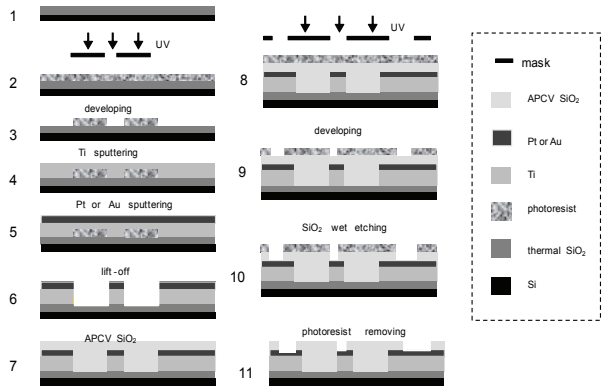

Fig. 2. Cross-sectional illustration of the electrodes, "lift-off" technology.

Standard photolithography and metal etching may be also applied to form such electrodes. This technological option is more complicated than the "lift-off" technique, especially because on the masking layer and etching parameters. Etching solution (containing thiourea, HCl, H_2O, H_2O_2) was used to etch the Au layer; chemical reaction:

$$Au + 2CS(NH_2)_2 \rightarrow Au(CS(NH_2)_2)_2^+ + e^-$$

and Ti layer was etched in the other chemical solution containing HF and H_2O_2. Thiourea (H_2NCSNH_2=$SC(NH_2)_2$) is a complexing agent for the gold dissolution in presence of appropriate oxidant - hydrogen peroxide, ferric ions, manganese dioxide or oxygen. This substance is non-toxic and not harmful for environment. Reaction and dissolution of the gold layer is fast enough to form thin-film electrodes. Pt electrodes were etched in the Ar plasma and then in HCl + HNO_3 8:1 chemical solution. Some experiments were also done with electrodes formation on the glass substrates, instead of the silicon wafers. Computer simulations with use of CoventorWare software were applied to estimate design rules for the devices as well as their technological, material and functional parameters.

Flow cytometry microsystem.

Laboratory demonstrator of the flow cytometry microsystem for detection of biological contaminations was developed and optimized. Research work was focused on the design, fabrication and testing of the portable device to detect selected bacteria cells with use of optoelectronic system, Fig. 3. In general in this system, the cells are marked by the antibodies labeled with fluorescent dyes and introduced from the preconcentrator chip [7], to the buffer fluid and central microchannel of the PDMS chip. Two side channels filled by the buffer fluid were applied to form the stream and focus the cells in a stream in the middle of the central channel. The fluid stream with both labeled and non-labeled cells passes through the region of optical detection. In this area the fluorescent dyes attached to cell surface is optically excited by the laser diodes: red (605 nm), green (532 nm) or blue (405 nm) and then fluorescence light is emitted by the dye. Since, wavelength of the emitted fluoroscence light by the dye-labeled antibodies attached on the cells membrane is shifted in respect to the excitation wavelength then it can be selectively detected thru emission filter by the photodetector.

The optical detection system consists of appropriate optical filters, lenses, optic fibers and detectors: avalanche photodiode (BPYP59), or photomultiplier (9658B). Sensitivity of both detectors should be sufficient to detect fluoroscence phenomena and to count the number of labeled cells passing through the central microchannel.

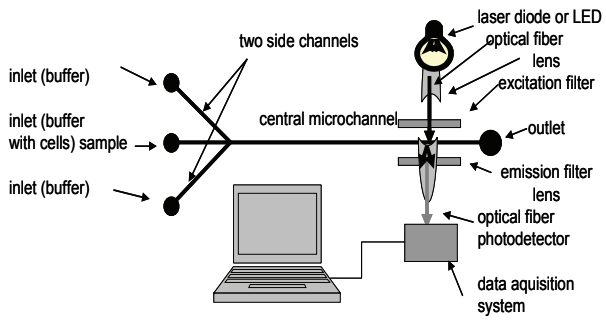

Fig. 3. Schematic arrangement of microfluidic and optoelectronic detection systems.

Computer design of the fluidic microchips arrangement was applied to fabricate photolithography mask and SU-8/Si replica templates. Microchannel replica shapes were 60 μm thick – this means, that microchannels were 60 μm deep. Standard ∅100 mm, 500 μm thick silicon wafer of arbitrary crystal orientation and electrical parameters were applied as the technological substrates. They were covered by the SiO_2 layer and 10-15 μm thick SU-8 photoresist layer to reduce PDMS adhesion to Si. The second 60 μm thick SU-8 layer was spinned-on and patterned with microchannel mask and processed with standard photolithography steps. PDMS molds were formed, cross linked and mechanically separated from the SU-8 templates. Molds were cut into the individual chips and fluidic openings to the microchannels were drilled with use of IR laser beam. Dummy PDMS chips, with flat both sides, were casted on the non-structured support. Surfaces of the both PDMS chips were activated in the oxygen plasma and bonded together to form microchannels, Fig. 4B.

Metal housing designed for the PDMS microfluidic chips was manufactured from dural PA5. It was intended to integrate fluidic elements with the optical detection system. Microfluidic chip was closed between two metal plates (Fig.

4C) with appropriate openings for external fluidic connections. Other housing elements were formed to enable optical access to the detection region of the microchannel – optical fibers connectors – one for the laser diode (or LED), lenses and optical filters, the second one for the photodetectors (photomultiplier or avalanche photodiode), lenses and optical fibers, (Fig. 4D, 4E). As the PDMS chip design include two symmetrical, separate and independent fluidic microchannel systems, the metal housing was specially designed to provide optical measurements with use of both microchannels with 180° rotation, only. To avoid overloading of the optical detectors by the excitation laser diodes, both optical fibers were oriented with the 45° angle to the PDMS chip surface.

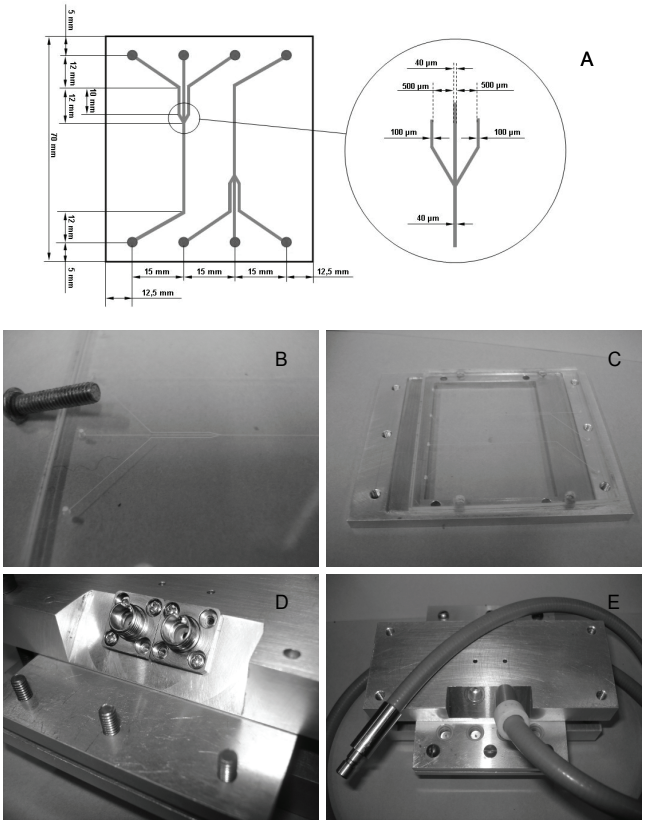

Fig. 4. (A) Layout of the microfluidic chip; (B) fragment of the PDMS microfluidic chip; (C) element of the metal housing with the PDMS microfluidic chip; (D) metal housing with 2 optical connectors to the optical fiber "pig tail" and laser diode; (E) metal housing with the fluidic connectors and optical fiber.

Conclusions

SU-8/Si templates, PDMS microfluidic chips, metal multi-electrodes silicon chips, PCB deep-sticks and optical detection system of the bacteria cells detection were manufactured. Several computer modeling and simulations were carried out to optimize devices geometry and evaluate functional parameters. Optimization of the system performance is directed towards the fluorescence signal improvement – proportion of the irradiation intensity and wavelength to the fluorescent marker parameters (wavelength) and detector sensitivity, response time [13]. Also microfluidic parameters of the detection system have to be optimized – microchannel dimensions, shape, flow rate of buffer in the central and side channels, optical fiber positioning in respect to the microchannel location, etc.

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REFERENCES

[1] Chang H-C., Yeo L.Y., Electrokinetically Driven Microfluidics and Nanofluidics, Cambridge University Press, 2010.

[2] Ilnicki F., Szczypiński R., Pijanowska D., Torbicz W., „Modelowanie ogniskowania hydrodynamicznego w mikrocytometrze przepływowym", XVI Krajowa Konferencja Biocybernetyka i Inżynieria Biomedyczna, 26-29.04.2010, Warszawa.

[3] Szczypiński R., Grzelka J., Baraniecka A., Lesiński J., Łysko J., Grodecki R., Pijanowska D., Grabiec P.: "Mikrocytometr przepływowy wykonany z PDMS z układem detekcji optycznej", Elektronika, 60, (2010) nr. 6, pp. 24-27.

[4] Szczypiński R., Grzelka J., Jabłońska-Kugler E., Ilnicki F., Baraniecka A., Lesiński J., Łysko J., Grodecki R., Pijanowska D., Grabiec P.: "Miniaturyzacja cieczowego cytometru przepływowego", XVI Krajowa Konferencja Biocybernetyka i Inżynieria Biomedyczna, Warszawa 26-29.04.2010.

[5] Szczypiński R., Grzelka J., Baraniecka A., Lesiński J., Łysko J., Grodecki R., Pijanowska D., Grabiec P.: "Cytometr mikroprzepływowy wykonany z PDMS z detekcją optyczną", XI Konferencja Naukowa (COE10), Nałęczów 20-23.06.2010.

[6] Baraniecka A., Rozum B., Dawgul M., Pijanowska D., Górska M., Dobrowolski R., Karbownik P., Domański K., Grabiec P., Łysko J.: "Microelectrodes for Amperometric Psychotropic Drug Detection", 10th Conference (ELTE10), Wrocław 22-25.09.2010.

[7] Baraniecka A., Szczypiński R., Grzelka J., Pijanowska D., Górska M., Grodecki R., Panas A., Grabiec P., Łysko J.: "Microfluidic Preconcentrator and Microfluidic Chip for Bacterial Cells Detection", 10th Conference (ELTE10), Wrocław 22-25.09.2010.

[8] Baraniecka A., Górska M., Zaborowski M., Prokaryn P., Szczypiński R., Skwara K., Szmigiel D., Sierakowski A., Nieprzecki M., Milczarek W., Łysko J., Pijanowska D., Grabiec P.: "Mikroprzepływowe immunoczujniki z detekcją amperometryczną", Elektronika, 50, (2009) nr. 12, str. 100-107

[9] Łysko J., Baraniecka A., Domański K., Grabiec P.: "MNS DIAG Project for the New Biochemical Microdevices Development", Science and Technology Days, 3rd Forum (STDF10), Białowieża 20-22.04.2010.

[10] Łysko J., Baraniecka A., Domański K., Grabiec P.: "Microfabrication for Biochemical Devices", Science & Technology Days, 3rd Forum (STDF10), Białowieża 20-22.04.2010.

[11] Łysko J., Pijanowska D., Baraniecka A., Grabiec P.: "Microfluidic Devices for Bio-Medical Applications", The 107th ICB Seminar (MNDB10), Warsaw May 13-15, 2010.

[12] Kazimierczak B., Baraniecka A., Łysko J., Dawgul M., Górska M., Dobrowolski R., Karbownik P., Kruk J., Torbicz W., Grabiec P., Pijanowska D.: "L-Ascorbic Acid Oxidation at a Thin-Film Platinum Electrode as an Indicative Reaction in Amperometric Immunosensors", 10th Conference (ELTE'10), Wrocław 22-25.09.2010.

[13] Grzelka J., Szczypiński R., Pijanowska D., Grodecki R., Lesiński J., Grabiec P., Łysko J.: "Optoelectronic Bacteria Cells Detection System", 10th Conference (ELTE'10), Wrocław 22-25.09.2010.

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