Sensors and filters based on nano- and microchannel membranes for biomedical technologies

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Abstract. A new technology is presented in a concise form which enables the silicon membranes to be produced over a wide range of channel dimensions from a few nanometers to tens of micrometers. There is good reason to believe that this method based on rather simple technical processing is competitive with other technologies for fabricating nanofluidic analysis systems. Some of the completed developments involving microchannel membranes, namely, the optical DNA-sensor and the human cell separation system are demonstrated without going into details. The other applications of micro- and nanochannel membranes, namely, the electrical sensor and electrokinetic filters for detecting and separating liquids and biomolecules are shown with the first results and are in progress.

1. Introduction
Genetic roots of diseases are the mainstream of current biomedical research and development. A new generation of fast, cheap sequencing technologies and nano- and microfluidic analysis systems is needed to achieve personalized medicine in the near future. Rapidly emerging developments in microfabrication technologies have enabled a variety of microfluidic systems consisting of valves, pumps, mixers, and reactors to be utilized effectively in biology and medicine for drug delivery, DNA analysis, and biological/chemical agent detection sensors. These microfluidic systems require integration of sample separation, collection, detection units with fluid pumping, flow control elements, and the necessary electronics on a single microchip [1].

Fluidic nanochannel structures have been also used for chemical and biomolecular sensing, separation of charged analytes, and other unique modes of molecular manipulations. These applications have been facilitated by the significant increase in the range of advanced nanofabrication techniques. Nanofluidic systems are fabricated using bulk and surface nanomachining based on electron beam and interferometric lithography or self-assembly patterning [2].

No technology for producing versatile membranes with channels the dimensions of which may be varied over a wide range however exists. This circumstance has threatened to retard biomedical research and development. In this paper we briefly describe a new approach to fabricate silicon nano- and microchannel membranes which can form the basis for advanced fluidic systems and can be
operated as “fluidic transistor”, optical DNA-sensor, electrical sensor, and as filters for mammalian cell separation.

2. Technology for producing membranes
A new technology of silicon nanochannel membrane fabrication has been developed. Conceptually the structure of these membranes comprises a monolithic connection of a nanochannel array with rigid supporting microchannel one and has an ordered profile of open through channels. Coupled with a fluidic system, the device which is performed in a monocrystalline silicon wafer is intended for separation, concentration, and detection of nanoparticles, cells, polynucleotide and protein molecules, their conjugates and associates [3-6].

The technology has been realized by two different techniques. In technique referred to as the “top-down” method, a nanochannel part of membranes is formed through series of plasmochemical and pyrolytical depositions of silicon and silicon oxide films directly on a microchannel array with overlapping microchannels. The lateral size of these microchannels can be decreased gradually from several micrometers to tens of nanometers, figure 1.

![Figure 1](image)

**Figure 1.** SEM images of the nanochannel membrane surface after different fabrication stages: (a) the starting microchannel membrane, (b) plasmochemical deposition of silicon film, (c) pyrolytic deposition of silicon oxide film – the final structure, (d-f) the separate channels.

In the “bottom-up” method, channels of microchannel array are sealed with SiO₂-nanospheres having a diameter of 50-100 nm. Then, a porous amorphous silicon film is plasmochemically deposited on the solid surface. After removal of silica plugs a molecular filter is obtained. This filter consists of the silicon film 40-400 nm in thick which is suspended over microchannels. Pore size in the amorphous silicon is less than a few nanometers. During subsequent processing these pores become through and expand in dimensions. Their diameter can be controllably increased to tens of nanometers, figure 2.
Figure 2. SEM images of the nanochannel membrane surface after different fabrication stages: (a) a porous silicon film deposited on silica plugs, (b) a fragment of the membrane over microchannel after removal of silica plugs, (c) a fragment of the membrane after electrochemical etching.

The thickness of nano- and microchannel parts of the membranes (length of the channels) is 0.1-6 µm and 100-250 µm, respectively. The lateral size of the channels changes in a wide range from a few nanometers to a few micrometers. Their surface densities are $10^5$-$10^8$ cm$^{-2}$ and $10^{10}$-$10^{11}$ cm$^{-2}$ for the 1-st and 2-nd methods, respectively.

The nano- and microchannel membranes made of biocompatible silicon by the present technology hold much promise for their integration with advanced electronic, optical, and biochemical devices.

3. Microchannel sensors

3.1. Optical DNA-sensor

We propose a simple optical method for hybridization analysis of DNA. This method is based on overlapping silicon microchannels with microspheres consisted of light scattering and light absorbing materials. DNA targets immobilized on the spheres bind them to microchannel surface due to specific hybridization reactions with complementary oligonucleotide probes covalently bound to an internal surface of this microchannel sensor. As a result, the registered intensity of transmitted light decreases, figure 3.
Figure 3. Transmission spectra of silicon microchannel matrix: 1) before and 2) after hybridization reactions, 3) the restored state of the matrix with oligonucleotide probes after denaturation of complementary complexes and removal of microspheres with DNA targets.

On the basis of the present method it becomes possible to develop a simple optical device for detection of DNA and other biological substances. This sensor can be designed of light source, microchannel matrix, photodetector, and several lenses. Multiplexing these elements on a silicon substrate enables a three-dimensional optical biochip to be constructed.

3.2. Electrical sensor
We have developed an electrical sensor based on microchannel semiconductor for liquid analysis. Silicon microchannel matrix acts as a sensitive element the conductivity of which changes specifically with the type of liquid coming to contact, figure 4.

Figure 4. Responses of the electrical sensor: (a) deionized water, (b) water-ethanol solution, 1:1; (c) aqueous solution of ammonium hydroxide, 18 w.%; fluid volume of 3 µl.

Specific and reproducible responses of the sensor for every kind of liquid make it possible to distinguish biological solutions and to register specific biochemical reactions in the interior of microchannels. Multiplexing these sensors on a silicon substrate permits an electrical biochip to be designed.

3. Human cell separation filters
A cell separation system is proposed which comprises a pile of the microchannel membranes with different channel lateral sizes of 2-30 µm. This microfluidic device is intended for size-selective and receptor-specific separation of viable rare cells circulating in human blood. In the second case, receptor specific antibodies are immobilized onto an internal surface of microchannel filter. It was found that the silicon membranes were easily permeated with even undiluted blood and did not damage human cells. This microfluidic system has provided its high efficiency for separating fetal cells from mother’s blood and tumour cells from blood of patients with breast cancer [7] or multiple
myeloma. The possibility to isolate tumour cells from patient’s blood is especially required for monitoring the tumour reoccurrence after therapy and our microfluidic device based on silicon microchannel membranes presents this opportunity, figure 5.

Figure 5. SEM images of oncotransformed HeLa cells separated from blood (in model experiment) on the surface of silicon microchannel filter with a pitch of 10 µm and channel openings of ~8.5 µm.

4. Electrokinetic silicon channel filters
Our silicon membranes provide a basis for separating and detecting different biological substances such as DNA, proteins, and cells around directed electrokinetic controlled transport of fluids and dissolved analytes through micro- and nanochannels. Using the silicon filters as a “fluidic” transistor, we can control over electroosmotic and electrophoretic flows by applying external biases to the semiconductor (as a “gate”) and biological solutions (as a “source” and a “drain”). This electrokinetic flow control scheme which does not require any moving components has been tested with profit to transport of buffered solutions, proteins, and CdS nanoparticles. The electroosmotic phenomenon in the silicon nano- and microchannel membranes has received much consideration in our experiments.

5. Conclusions
The technology has been developed which enables the silicon membranes to be formed over a wide range of channel dimensions from a few nanometers to tens of micrometers. We believe that this new method based only on simple technical processing is quite competitive with present-day technologies for production of nanofluidic analysis systems controlled with low-driving electric fields.

This technology has been applied advantageously to the completed developments involving the microchannel membranes, namely, the optical DNA-sensor and the cell separation system. The other applications of micro- and nanochannel structures for the electrical sensor and electrokinetic filters are still being developed.

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