A microfluidic device for impedance spectroscopy analysis of biological samples

Ciprian Iliescu\textsuperscript{a,}\textsuperscript{*,}  Daniel P. Poenar\textsuperscript{b,1}, Mihaela Carp\textsuperscript{b,1}, Felicia C. Loe\textsuperscript{a,2}

\textsuperscript{a} Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669, Singapore
\textsuperscript{b} Microelectronics Centre, School of Electrical & Electronic Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

Received 23 May 2006; received in revised form 3 August 2006; accepted 12 August 2006

Available online 14 September 2006

Abstract

The paper presents an original fabrication process of a microfluidic device for identification and characterization of cells in suspensions using impedance spectroscopy. The device consists of two glass wafers: a bottom wafer comprising a microfluidic channel with two electrodes added for impedance measurement, and a top glass wafer in which inlets and outlets are realized. The fact that the device is glass-based provides a few key advantages: reduced influence from parasitic components during measurements (due to the good isolation properties of the substrate), optical transparency and hydrophilic surface of the microfluidic channel. The latter feature is especially important as it enables sample suction due to capillarity forces only. Thus, no external pumping is required and only a small volume sample suffices for the measurement.

The fabrication process of this device consists of three major steps. First, via-holes and inlet/outlet holes are executed in the top glass wafer by wet etching in a 49\% HF solution using a low stress amorphous silicon/silicon carbide/photoresist mask. Second, the microfluidic channel is etched into the bottom wafer and Ti/Pt electrodes are then patterned on top of it using a spray coating-based lithography. The last processing step is bonding together the top and bottom glass wafers by employing a very thin adhesive intermediate layer (SU8). This adhesive layer was applied selectively only on the bottom die, from a Teflon cylinder, using a contact imprinting method.

Finally, devices with three different electrode geometries were successfully tested using DI water, phosphate buffer saline (PBS), and both dead and living yeast cells resuspended in PBS. Clear differences between dead and live cells have been observed.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Microfluidic chip; Impedance spectroscopy; Adhesive bonding; Wet etching of glass

1. Introduction

Over the last decade, the investigation of cell systems has significantly increased in volume and importance and has become a necessary tool in different fields, such as biotechnology, pharmacy, biomedicine, food and environmental technologies. However, in such cases cell immobilization is routinely used for various analyses or applications, e.g. wastewater treatment, production of biopharmaceuticals and fermentation of beverages [1,2]. The most widely used immobilization methods are based on living cells entrapment in various media such as carrageen, alginate, agar, polyvinyl alcohol, sol–gel, etc. [3–5]. Thiolated Salmonella antibody immobilization onto an Au-covered quartz surface enabled fast and sensitive detection of food poisoning [6]. Yeast cells (Saccharomyces cerevisiae) have also been immobilized on a self-assembled monolayer (SAM) of alkanethiolate on Au electrodes for electrochemical impedance spectroscopy (EIS) measurements [7].

However, the requirement of immobilization of a certain bio-component (e.g. of an antibody) necessary to capture the cells on the surface, although less time-consuming than the classical microbiological culture methods, still remains a cumbersome technique which requires a relatively complex procedure of preparation before the actual usage of the realized device. For this reason, faster detection methods are desired.

Electrochemical impedance spectroscopy is a well-established method which has been applied in many fields of science and technology [8]. Furthermore, in the last decade, EIS has also been used extensively in biochemical and medical
applications, as it has proven to be a sensitive technique to
detect and measure various biochemical or biological events.
For example, it can be used for sensing the formation of
antigen–antibody complexes [9], immunosensing [10], DNA
characterization [11,12] or detection of DNA hybridization
[13], as well as characterizing living cells: both quantitatively
and qualitatively bacteria impedance measurement of $10^2$
colony-forming-units per ml (cfu/ml) with only a 15 min
diagnosis time has been reported [14]. Thus, the EIS technique,
combined with microfluidics, micromachining and MEMS
techniques, demonstrated that it can be a very useful and
valuable tool in biochips for easy and fast characterization
of bio-samples, which is especially important as until now
few automated methods exist for such purposes. Moreover,
another essential merit of EIS is that it is label-free, therefore
simplifying even further the preliminary sample preparation for
analysis.

Other previous realizations include glass-based chips with
either multiple separate Pd electrodes [15] or a set of interdig-
itated ITO electrodes [16] with which chromaffin or E. coli
O157:H7 cells were characterized or detected, respectively.
Microscale impedance-based techniques were also used in Si-
based chips, not to characterize the cells per se but to detect the
variations in the suspension’s impedance caused by the release
of ionic species by metabolizing cells [17,18]. EIS can also be
applied for relevant biomedical applications, such as the detec-
tion of cancerous tissue cells from normal ones [19,20].

We report in this paper the realization of a simple but very
effective microfluidic device for EIS-based identification and
characterization of cells. The device was fabricated in glass
and consists of two dies that are bonded together using a thin interme-
diate adhesive layer (SU8). The microfluidic channel is etched
in and the necessary electrodes are then deposited on the bottom
die. The second die, in which inlet and outlet holes had previ-
ously been etched, forms the top lid. In contrast with previously
reported results, we employ an innovative adhesive bonding
method using contact imprinting that allowed us to overcome
the problems due to the bottom wafer’s surface nonplanarity.
The realized devices have been tested using suspensions of vari-
ous types of living and dead cells suspended in buffer, as well
as pure buffer and DI water. Another essential feature of our
device distinguishing it from other previous realizations is that
no external pumping is required to feed through the analyzed
sample. Instead, a simple droplet placed unto the inlet open-
ing was slowly sucked in the microfluidic chamber by capillary
forces due to the hydrophilic surface of the microfluidic channel.
Finally, our devices can easily be re-used after measurement if
a thorough cleaning procedure is executed.

2. Device structure and layout

For a good functionality of the device we chose glass as the
main material for its fabrication. It is well known that glass
presents good dielectric properties in a wide frequency range
(for Corning 7740 – the glass used in our fabrication process –
the dielectric constant at $20^\circ$C and 1 MHz is 4.6). Additionally,
by using glass the device will be optically transparent and the
position of the cells can be very easily observed using classical
or confocal microscopy. From the fluidic point of view, glass is
also advantageous as it presents a hydrophilic surface, a very
important aspect for a microfluidic device. Finally, glass is a
well known material for its biocompatibility.

The structure of the device, shown in Fig. 1, consists of two
glass dies separately processed that are finally bonded together.
The inlet/outlet holes for the cells suspension solution and two
via-holes for the electrical contacts are patterned in the top
glass die, whereas the microfluidic channel and the electrodes
are realized on the bottom one. A metal film is deposited on
the top surface of the bottom wafer and follows its relief pro-
file, so that electrodes are realized not only at the bottom of
the microfluidic channel but also on its walls. Fig. 2 shows a
schematic representation of the electrodes design. As it can be
seen, three different types of electrodes have been investigated:
interdigitated (Fig. 2a), parallel (Fig. 2b) and circular (Fig. 2c),
respectively. When the introduced cell suspension reaches the
measurement region with the electrode structure it will cause an
impedance change between these electrodes depending on the
number of cells, their characteristics (complex permittivity)
and the applied frequency. Therefore, this device can be efficiently
used for cell identification and electrical characterization.

3. Fabrication

The fabrication of the device consists of three major steps:
realizing etch-through holes in the top glass wafer, processing
the microfluidic channel and the associated electrodes on the bot-
tom glass, and bonding together the two glass wafers followed
by dicing.

3.1. Fabrication of the top glass wafer with etch-through
holes

In most of the reported realizations, the inlet/outlet holes
were realized using classical techniques such as: drilling with
diamond bites [21], ultrasonic drilling [22], electrochemical dis-
charge [23] or powder blasting [24]. In this case, a much more
convenient and easy to use micro fabrication technique was
employed. This technique employs wet etching in a HF solution,
which, if well designed and carefully executed, can be easier to
implement as well as much more versatile and more effective
than the previously mentioned methods. The main steps of the
fabrication process for the etch-through holes in the top wafer are
presented in Fig. 3. The process started from blank Pyrex glass
(Corning 7740) wafers, because this type of glass has a com-
position with a low concentration of oxides that provide insoluble
reaction products after reacting with HF. A 4 in. glass wafer
500 μm thick was used for the top die (Fig. 3a). The wafer was
cleaned in a classical piranha solution (H₂SO₄ + H₂O₂ 2:1) at
120 °C for 20 min, followed by rinsing in DI water and spin-dry.

For masking purposes a low stress amorphous silicon/silicon
carbide/photoresist tri-layer coating was used. The first 2.5 μm-
thick amorphous silicon (α-Si) layer was deposited on a PECVD
system (STS, Multiplex PRO-CVD) at 300 °C, a pressure
of 900 mTorr, a power of 500 W (high frequency mode at
13.56 KHz) using a SiH₄/Ar gas mixture with gas flows of
120 sccm/700 sccm, respectively. It is important to outline that
achieving a low stress in the α-Si layer is desired in order to
obtain a better performance of the tri-layer composite masking
coating. The overall average residual stress in the as-deposited
α-Si masking layer was 530 MPa (compressive) when mea-
sured using a method previously described in literature [25], but
reduced significantly to 130 MPa (compressive) after an anneal-
ing process (400 °C/3.5 h).

In order to both increase the etching resistance towards HF
and to further reduce the overall stress, a second layer of PECVD
silicon carbide (SiC) 0.5 μm thick was deposited in the same
equipment used for the deposition of the previous α-Si layer.
The SiC deposition was performed at 300 °C, at a pressure
of 1100 mTorr and a power of 150 W, using SiH₄ (45 sccm), Ar
(700 sccm) and CH₄ (310 sccm). The overall residual stress in
the composite α-Si/SiC masking coating after the SiC deposi-
tion was around 50 MPa (compressive). For the patterning of
this α-Si/SiC masking coating a 2 μm-thick positive photoresist
(AZ7220 from Clariant) was used. After patterning the photore-
sist layer a hard baking process on a hot plate at 120 °C for
30 min was performed in order to increase the adhesion of the
photoresist on the SiC layer. The patterning of the composite
masking coating (Fig. 3b) was performed in an ICP DRIE equip-
ment (AMS100-DE from Adixen) using SF₆ (300 sccm) at a coil
power of 2000 W and a platen power of 100 W. A dummy silicon
wafer was bonded with wax on the back of glass wafer (Fig. 3c)
to prevent the etching of the opposite side of the wafer.
The wet etching process for patterning of inlets/outlets was then performed in a 49% HF solution for 85 min with magnetic stirring (Fig. 3d). The etching process was stopped on the wax layer. The result of the etching is presented in Fig. 4, which shows the etched wafer with its masking layer still on it. It can be easily noticed that the etching process provided excellent results, and that no pinholes were present in the masking coating. The removal of the $\alpha$-Si/SiC/photoresist composite coating (Fig. 3e) was performed in the same ICP deep RIE system using $O_2$ and $SF_6$, respectively, with a similar recipe as the one previously used for patterning the same tri-layer masking coating.

The separation of the glass and silicon wafers (Fig. 3f) was performed on a hot plate at 100 $^\circ$C. After debonding the glass wafer was cleaned in piranha at 120 $^\circ$C rinsed in DI water and spun-dry.

3.2. Fabrication of the bottom glass wafer with the microfluidic channel and electrodes

The main steps of the fabrication process of the bottom glass are presented in Fig. 5. The same type of glass (Corning 7740) wafer was also used for the bottom die (Fig. 5a). After the cleaning process, a 1.2 $\mu$m-thick $\alpha$-Si layer was deposited, using the same procedure described previously. The patterning of the masking layer was also performed with a similar process as that used for the fabrication of top glass wafer. The etching of the microchannel was performed with a 49% HF solution with magnetic stirring in a Teflon container for 3 min, resulting in trenches about 25 $\mu$m deep (Fig. 5b). After stripping off the photoresist layer in a NMP resist stripper, the $\alpha$-Si layer was also removed in a 30% KOH solution at 90 $^\circ$C (Fig. 5c). After a classical cleaning process, Ti/Pt (50 nm/150 nm) layers were deposited by sputtering using a Nanofilms equipment (Fig. 5d). One critical step of this fabrication process is the patterning of the Ti/Pt metallization. Due to the nonplanarity of the device (the microchannel is 25 $\mu$m deep) the classical spin-coating based deposition of the photoresist cannot be successfully applied. For this reason a spray coating process is recommended, and in this case an EVG101 spray coating system was used, employing an AZ4620 photoresist (from Clariant). The resist was diluted with MEK and PGMEA to a suitable viscosity, in order to achieve a better coverage of the walls and the edges of the etched trench. After a series of investigations that are reported in detail elsewhere [26], it was observed that the optimum ratio for the AZ4620:MEK:PGMEA mixture was 1:1.5:0.5. With this solution, good coverage and uniformity of photoresist are achieved not only on planar surfaces, but also on the sidewall of the etched trenches. The thickness of the photoresist film was 5 $\mu$m. After patterning the photoresist (Fig. 5e), the etching of the metal layer was performed by “back sputtering” in a Unaxis–LLS EVO system, using Ar at a pressure of $8 \times 10^{-4}$ mbar, and a RF power of 300 W (Fig. 5f). After the etching of the metal layer the photoresist was removed in the resist stripper.

3.3. Assembly of the glass wafers

The assembly of the top and bottom wafers is a critical step in the fabrication of the final microfluidic device. On the one hand, it is desired that all surfaces in direct contact to the fluids should be hydrophilic and for this reason the realization of the microfluidic channel in hydrophobic materials (such as SU8 or BCB) is not recommended. On the other hand, the application of glass-to-glass bonding is also limited due to two main reasons: first, the nonplanarities of the wafer caused by metal layers may impede a good bonding, and secondly, such a process must be carried out at high temperatures which may be incompatible with the already existing metallic layers.

Therefore, in this case we have employed adhesive bonding, as it offers some important advantages such as low temperature processing (up to 200 $^\circ$C) and insensitivity to small nonplanarities of the surfaces to be bonded. For this specific application a special adhesive bonding method was developed consisting of
imprinting an adhesive layer on the top surface of one of the glass wafers (typically the bottom one). In general, the printing must be carried out unto the wafer which has the smallest area of flat top surface as this factor dictates the definition of the bonding surfaces between the two wafers. After the printing step, the bonding process was carried out in a classical wafer bonding equipment EVG-520. For the adhesive layer a common negative photoresist SU8-5 was selected. The entire adhesive printing method is described in Fig. 6. First a thin SU8-5 layer is spun on a 6-in. dummy silicon wafer which was previous treated in a HNO₃ solution in order to render its improve surface hydrophilic (Fig. 6a). The SU8-5 layer is transferred on a Teflon cylinder (Fig. 6b) by rolling the cylinder on the surface of the dummy wafer. After the adhesive layer is transferred on the cylinder it can be easily imprinted on the bottom wafer surface using the same method (Fig. 6c). The wafers were aligned manually on a wafer bonder frame and bonded in the wafer bonding equipment EVG-520 under vacuum, at 150 °C, for 30 min with an applied pressure of 1000 N (Fig. 6d).

The measured thickness of the SU8-5 layer after bonding was between 1.5 and 3.5 μm as observed under an optical microscope. For the adhesive bonding process using contact imprinting, a critical parameter is the quantity of adhesive material (SU8-5 in this case) that is imprinted on the bonding surface. A too large quantity of adhesive applied on the surface increases the amount of the same material that will be later reflown in the microfluidic channel. This phenomenon is presented in Fig. 7a, where a planar glass wafer was bonded on a silicon wafer with channels 20 μm deep. It can be noticed that the wall of this channel is made from the adhesive that has reflown in the microfluidic channel initially etched in the glass wafer. In contrast, an optimal process that resulted in excellent results is presented in Fig. 7b, where no adhesive reflow accompanied the bonding between the two glass dies.

There are two main methods to reduce this adhesive reflow effect. The first one is to reduce the thickness of the adhesive layer applied onto the bonding surface. This can be achieved by using an adhesive with a low kinematic viscosity, such as SU8-5 (265 cSt), so that a thin adhesive layer can be deposited on the dummy wafer. A second method consists of reducing the bonding area. Introducing elevated ridges 0.5 mm wide in the chip design around the microfluidic channel will reduce the contact area and therefore minimize the quantity of adhesive that will flow in the etched channel during the bonding process.

After bonding the wafer was diced using DISCO 3350 equipment. The result of the fabrication process is presented in Fig. 8.

4. Testing and results

The testing of the device was performed with DI water, phosphate buffer saline (PBS) - for the reference measurements - and with suspensions of dead and living cell populations extracted from their original cultures and re-suspended in PBS. For example, the suspension of living yeast (Saccharomices cerevisiae) cells was prepared starting from 50 mg of yeast, 50 mg of sugar and 1 ml DI water, which were mixed and then incubated in an
Eppendorf tube at 37 °C for 2 h. The cells were then concentrated by centrifugation at 1000 rpm for 1 min. The supernatant solution was removed and the cell pellet was washed by adding 2 ml of DI water into the tube. The centrifugation and washing process was repeated three times before the cells were collected and the batch was divided into two. The first part was resuspended in PBS and used for the characterization of the existing living cells, whereas the second part was resuspended in DI water and boiled for 1 h in order to obtain the desired suspension of dead cells. The resulted concentration was $1 \times 10^7$ cells/ml. The electrical characterization was carried out using a probe station and a Solartron 2160 impedance analyzer. The devices were tested in the frequency range between 1 kHz and 1 MHz.

The test results obtained for DI water and PBS (both tested as possible references), dead and living yeast cells are presented in Figs. 9–11 for devices with interdigitated, parallel and circular electrodes, respectively. One can immediately notice the different results provided by the three structures. Particularly the device with circular electrodes provides a clearly different response compared to those of the devices with interdigitated and parallel electrodes.

Fig. 9. Measured data for the dependence on frequency of the impedance (a) and phase (b) for DI water, and dead and living yeast cells suspensions, respectively. The results of this EIS analysis were obtained using a fabricated microfluidic chip with interdigitated electrodes.

Fig. 10. Measured data for the dependence on frequency of the impedance (a) and phase (b) for DI water, and dead and living yeast cells suspensions, respectively. The results of this EIS analysis were obtained using a fabricated microfluidic chip with parallel electrodes.

Fig. 11. Measured data for the dependence on frequency of the impedance (a) and phase (b) for DI water, and dead and living yeast cells suspensions, respectively. The results of this EIS analysis were obtained using a fabricated microfluidic chip with circular electrodes.
In order to explain at least partially these differences, one must remember that such a measurement structure is in fact an electrochemical cell and more phenomena contribute to the operation of the entire measurement structure: the double charge layer and electrochemical phenomena at the electrode–electrolyte interface, the impedance of the cells and the impedance of the buffer in which the cells are suspended. As the last two elements were present in all three cases, only the first group of factors can be the cause for the observed differences. The entire measurement structure can be considered as an electrochemical cell, whose overall equivalent circuit is presented in Fig. 12 [27]. It consists of a series resistance $R_S$, the double layer’s capacitance $C_{DL}$, the charge transfer resistance $R_{CT}$ and the Warburg impedance $Z_W$ which models the ‘resistance’ to mass transfer. Assuming a standard one-step one-electron process $O + e^{-} \rightarrow R$, the Warburg impedance can be expressed as [27]:

$$Z_W = \sigma \sqrt{\frac{2}{\omega}},$$

(1)

where

$$\sigma = \frac{RT}{F^2A\sqrt{2}} \left( \frac{1}{c_O \sqrt{D_O}} + \frac{1}{c_R \sqrt{D_R}} \right)$$

(2')

with $F$ is the Faraday constant; $A$ the electrode surface area; $c_{O,R}$ concentration of species O and R, respectively; $D_{O,R}$ diffusion coefficients of species O and R, respectively; $R$ the universal gas constant; $T$ is the temperature. This expression can be re-written in a different form for the special case when the diffusivities and the concentration of the two species are equal, and when $n$ electrons may be implied in the reaction:

$$\sigma = \sqrt{2RT} \cdot \frac{1}{nF^2A} \cdot \frac{1}{c \sqrt{D}}$$

(2'')

Then, the total impedance of the electrochemical cell can be calculated, and its real and imaginary components are given by the relations [28]:

$$\text{Re}Z = R_S + \frac{R_{CT} + \sigma \omega^{-1/2}}{(C_{DL} \sigma \omega^{1/2} + 1)^2 + \omega^2 C_{DL}^2 (R_{CT} + \sigma \omega^{-1/2})^2}$$

(3')

$$\text{Im}Z = \frac{\omega C_{DL} (R_{CT} + \sigma \omega^{-1/2})^2 + \sigma \omega^{-1/2} (\omega^{1/2} C_{DL} \sigma + 1)}{(C_{DL} \sigma \omega^{1/2} + 1)^2 \omega^2 C_{DL}^2 (R_{CT} + \sigma \omega^{-1/2})^2}$$

(3'')

and of course, the total impedance magnitude will be $|Z| = \sqrt{(\text{Re}Z)^2 + (\text{Im}Z)^2}$.

One of the main reasons of pursuing miniaturization of electrochemical systems, like the proposed chip for EIS measurements, is that the down-scaling in size will provide increased sensitivity. Indeed, if we consider the measurement sensitivity as $S_Z = \partial |Z|/\partial A$, then its variation with the applied signal frequency results as shown in Fig. 13, with the electrode surface area $A$ as a variable parameter [28].

It is clear that smaller electrodes can provide improved sensitivities. However, it must not be forgotten that the magnitude of the useful signal also depends on the area of the electrode. Hence, reducing the size of the electrode could also reduce the signal-to-noise ratio and thus worsen the overall performance.

However, the differences between the results obtained using different devices cannot be due solely to their different electrode areas exposed in the measurement chamber. They are also influenced by the shape of the electrodes, as this determines the distribution of the electric potentials in the measurement chamber. It is obvious that the parallel electrodes would generate a quasi-uniform electric field between them, whereas a strongly non-uniform electric field with curved field lines normal unto the circumference of the electrodes will be present in the device with circular electrodes. This feature, also combined with the more localized positioning of the electrodes in the measurement chamber, may make the performance of the latter type of structure very dependent on the exact position of the cells with respect to the electrodes. Hence, for suspensions of low cell concentrations a large variability of the results is to be expected for different measurements executed with the same device even at the same concentration, resulting in a reduced reproducibility. In comparison, the interdigitated electrodes may provide a larger electrode area, and a non-uniform electric field will still be present, but are limited only to the corner regions. However, the way in which the electric field lines fill in the volume of the measurement chamber will be very different. Thus, the presence of sharp corners (or of other layout features leading to the crowding of the electric field lines) and the relative area they occupy with respect to the total electrode surface area is another cause for the difference between displayed results.
Additionally, the perimeter/area ratio may also play a role: the electric field lines may follow the contour of the electrodes, but the exposed electrode area determined the total current flowing through the entire chamber.

The results shown in Figs. 9–11 demonstrate that care must be taken when comparing previously reported results that are obtained with different devices having various internal electrode topologies. It is clear that one-to-one comparisons cannot be easily done, unless the shape and all the other parameters of the electrodes and measurement chamber are exactly identical.

The difference between the PBS and live/dead cells curves (for all three devices—Figs. 9–11) indicates the clear influence of the suspended cells (live or dead) on the impedance characteristics.

Finally, it must be highlighted that the chip is a very cost-effective solution, as it allows repeated re-usage, after a thorough cleaning procedure. Multiple measurements could be performed if after each measurement the device was first cleaned with DI water in an ultrasonic system and then dried in an oven at 120 °C, if necessary repeating this cycle more times. The cleaning of the device after each experiment was a very important factor in achieving repetitive results under the same conditions. The quality of the cleaning process was confirmed by plotting the characteristic of the device using air as media before each measurement. Any alteration of the characteristic indicated that the device’s measurement chamber was not sufficiently clean and, hence, the cleaning and measurement procedure was iteratively repeated until the initial impedance value of the device with air was achieved again. In this respect, related to the importance of the cleaning procedure, it must also be highlighted its great relevance for the reproducibility of the measurement results.

We noticed that the presence of any parasitic particle even dust, attached unto the electrodes or flowing through the measurement area, can affect negatively the measurement quality (especially if the size of the particle is comparable to the dimension of the electrodes, i.e. 30...50 μm). Also any parasitic deposition of unwanted thin films on the electrodes can modify the results. Finally, it is crucial to have a measurement setup that must be rigid enough and very carefully configured so as to avoid or minimize the contribution of unwanted parasitic elements.

5. Conclusions

The paper presented the successful fabrication process of a glass-based device for EIS characterization of cells, but quite obviously the design of the device can be easily modified to be applicable to other types of bio-components, too. Three innovative methods were used in the processing of this device: first, inlets and outlets were realized entirely by HF-based etching through the top wafer, employing a multilayer masking coating. Secondly, spray coating-based patterning of the electrodes was used as they had to follow the contour of the microfluidic channel. Thirdly, a special adhesive bonding method was developed consisting of imprinting an adhesive layer of SU-8 from a roller, followed by thermal bonding.

The fact that the microfluidic device is fabricated in glass confers it some key advantages such as: EIS analysis in a wide range of frequency spectrum, transparency for ease of observation using classical and/or confocal microscope, and hydrophilicity of the microfluidic channel. This latter feature is especially important as it causes self-driving capillary-based movement of the measured sample within the device to the measurement area. Therefore, because pumping is no longer necessary, only a very small volume droplet can be sufficient for the analysis. The device was successfully tested on DI water, PBS, live and dead yeast cells. The fabrication process can be applied also for fabrication of other microfluidic devices such as dielctrophoretic or electrophoretic devices.

Acknowledgements

This work was carried out as part of the “BioMEMS for Cells Characterization” project (code no.: 022 107 0008), whose funding by A*STAR is gratefully acknowledged by all the authors. The work in IBN was developed under the project IBN/04-R44007-OOE. We also wish to thank both present and former project members, especially Dr. Eishi Igata for his contribution in the impedance sensitivity calculations, and to the Sensors & Actuators laboratory staff of EEE-NTU for their support during the experiments.

References


Biographies

Ciprian Iliescu received BS and PhD degree from School of Mechanical Engineering, Polytechnic University of Bucharest in 1989 and 1999, respectively. While pursuing his PhD degree he worked at Baneasa S.A. where he was involved in design and fabrication of pressure sensors. Between 2001 and 2003 he was with Micromachines Center, Nanyang Technological University, Singapore. Currently, he is senior research scientist at the Institute of Bioengineering and Nanotechnology. His current research projects are related to dielectrophoresis, electrical characterization of cells and transdermal drug delivery. Dr. Iliescu is co-author of more than 100 papers published in journals and conferences proceedings.

Daniel Puiu Poenar received the MSc degree in electronics and telecommunications in 1989 from Polytechnic University, Bucharest, Romania. He received the PhD degree from the Technical University Delft in 1996. He then joined the Institute of Microelectronics (IME) Singapore, as a research engineer until 1999. Since 2000 he has been an assistant professor with the Electrical and Electronics Engineering (EEE) School, Nanyang Technological University (NTU), Singapore. His research interest are MEMS and micromachining and their application for the development of sensors and actuators, especially for (bio)chemical devices. Dr. Poenar is co-author of more than 40 papers published in journals and conferences proceedings.

Felicia C. Loe received BS in biochemistry from University of Wisconsin, Madison, in 2003. Currently, she is pursuing her PhD at the National University of Singapore. Her current research projects are related to cell phenotype modulation and tissue engineering.

Mihaela Carp received her MSc degree in physics from University of Bucharest, Romania in 2001. Since 2003 she has been working as a PhD student in the EEE School of NTU, Singapore. In 2005, she joined the bioMEMS research group led by Assist. Prof. Poenar Daniel. Her main research interests are development of novel colour sensors as well as design, simulation, fabrication and characterization of microfluidic devices for biomedical applications.