BioMEMS for Mitochondria Medicine

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1 ABSTRACT

We designed and fabricated BioMEMS for monitoring complementary parameters important in mitochondria medicine. BioMEMS were first simulated using COMSOL Multiphysics 3.4 and then fabricated using silicon technology to produce an SU-8 based microfluidic system. We used arrays of gold electrodes for electromagnetic interrogation, Ion Sensitive Field Effect Transistors (ISFET) for measurements of pH, Na, Ca, and K ions, flow cytometers for optical measurements and an (20μmx20μm) impedance cytometer for dielectric spectroscopy. Electrodes were designed as 2- and 4-probe structures, for optimized operation at high frequency and for low frequency, respectively. In addition, to limit polarization effects at low frequencies, we have one 4-electrode set-up with two planar external electrodes and meshed internal electrodes.

Keywords: BioMEMS, mitochondria, impedance spectroscopy, cytometry.

2 INTRODUCTION

Mitochondria are the cellular powerhouse that produce chemical energy source, ATP in a process of oxidative phosphorylation. However, they also play critical role in various specialized metabolic functions (signaling, synthesis, etc.) related to specific cells where they reside. ATP is produced in an inner membrane by proteins called electron transport chain (ETC), which pump out protons from the matrix to the intramitochondrial membrane space leading to synthesis of ADP and inorganic phosphate Pi to ATP while consuming oxygen. Important parameters of this process that control ATP production and help in its characterization are proton gradient and membrane potential. Dysfunction of these processes as well as other mitochondria functions [1], [2] became an object of great interest in fight against many diseases, such diabetes, neurological diseases, heart failure etc., as well as aging, where mitochondrial dysfunction may occur many years prior to symptoms. Diagnoses of mitochondrial disorders rely mainly on biochemical and morphological methods but also on direct measurements of different chain complexes, optical tests, and patch- and voltage-clamp techniques.

Due to the great complexity and multitude of mitochondrial dysfunctions, multiparametric approach is needed, such as possible in lab on a chip, to capture all intercorrelated processes and effects. We intend to focus on noninvasive or minimally invasive measurements, possible by capacitive coupling of electric field through the plasma membrane in our electronic sensors to allow for monitoring processes related to mitochondria disfunctionality. Along with measurements of oxygen and gradient of proton concentrations, these include measurements of membrane potential [3] acquired by E-fields [4], which in turn are controlled by electrodes' configuration, amplitudes, and frequency of the signals. Large E-fields, $\sim 3 \cdot 10^7$V/m, are present at the inner membrane of mitochondria and in addition, small dimensions of the devices used as probes produce high fields further putting constraints on measurements interpretation. In designing a sensor for impedance measurements, low impedance interface between electrode and electrolyte is important [5], especially for very low signal levels (μV- mV).

3 FABRICATION AND SIMULATION

3.1 Flow Cytometers

Flow cytometers for characterization of particles in a fluid were designed to measure parameters of the mitochondrial membrane using fluorescent probing and also dielectric spectroctroscopy. Microfluidic cytometers have been developed using a single stage of focusing [6] enabling the study of one cell at a time. Flow cytometry is valuable in determining the mitochondrial membrane potential [7] by means of fluorescent probes such as TMRE

(tetramethylrhodamine-methylester). Hydrodynamic focusing is typically employed with the analyte flowing within a focusing fluid shield. In order to accommodate various biosamples' sizes we designed cytometers of different channel widths: 30 µm, 15 µm, and 20 µm, which in addition includes sidewall electrodes for impedance spectroscopy. Two stages for fluid focusing improve alignment and collimation, while by preventing diffusion of the analyte they increase the working range for analysis. The side scatter of the applied laser is collected at 90 degrees to the incident laser light and provides information from fluorescence labeling of biosamples.

The MEMS microfluidics module of Comsol Multiphysics 3.4 was used to simulate (Navier-Stokes equations) the flow in the different cytometers following (Fig. 1 and Fig. 2).

The first cytometer with a width of 30 µm has four focusing inlets. Simulation of the fluid flow using inlet analyte concentration of 50 mol/cm^3 and pressure of 50 psi shows long range at the focusing distance of about 10 µm (Fig 1). The second flow cytometer with the 20 µm channel uses one focusing inlet and gets focused to about 5 µm with less collimation.

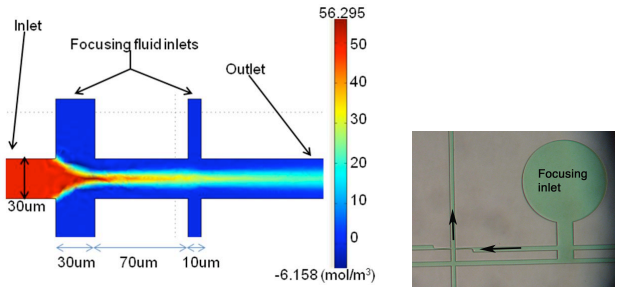

Figure 1: Comsol simulation of the concentration distribution and the fabricated structure.

An impedance cytometer was designed to perform dielectric characterization of cells/mitochondria as a function of frequency to obtain information about their membranes [8]. It has a 20 µm wide channel, with 10 µm wide focusing fluid inlets (Fig 2), where cells pass consecutively at vertical electrodes embedded in the side-walls for impedance measurements. Four electrode pairs were done by elaborate thick electrodeposition of gold (Fig 3) to produce uniform E field, unlike that of conventional planar thin film electrodes. Electrode height smaller than the channel should be avoided as it produces non uniform fields, thus distorting measurements [9]. Impedance measurements as a function of frequency require very sophisticated electronics for broad band spectroscopy using maximum length sequences, as well as signal processing but can deliver information on dielectric properties of biosamples, especially on membrane capacitance [10], [11]. Furthermore, incorporation of electrodes in cytometry allows for comparison of optical measurements used to identify membrane potential with corresponding electrical responses and address the issues of dye role in accuracy.

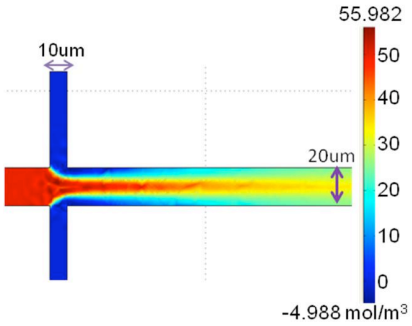

Figure 2: Comsol simulation of concentration distribution.

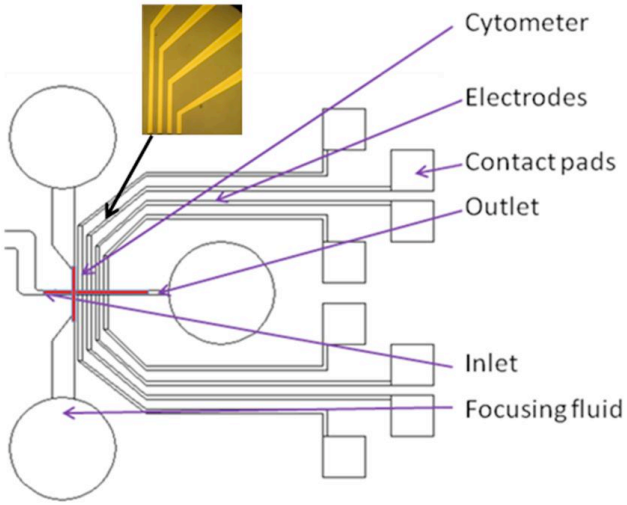

Figure 3: Schematic of flow cytometer along with the side wall electrodes.

All the cytometers as well as other microfluidic channels were fabricated using silicon technology and SU8 photoresist to form the channel walls. The SU8 was patterned by photolithography.

3.2 Electrode Setup

Electrode arrays in fluidics were implemented for impedance spectroscopy. The dielectric properties of cells at low frequencies depend on the membrane properties, and enables measuring the membrane potential in a non-invasive manner [12]. To limit electrode polarization, which is caused by ionic accumulation at the electrode - electrolyte interface and dominates the system at low frequencies due to its capacitive behavior, especially for of biological samples, we used four electrodes configuration (50 µmx600 µm with 100 µm spacing) to take advantage of high input impedance of the amplifier. Pairs of internal electrodes were designed either as planar or meshed structures with the square holes 7.5 µm by 7.5 µm in size (Fig 4). At higher frequencies ~ 20 kHz [13], beyond electrode polarization range, we use 2- probe configuration where the width of 50 µm was selected [14] to lower their parasitic resistance.

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1782-7 Vol. 1, 2009

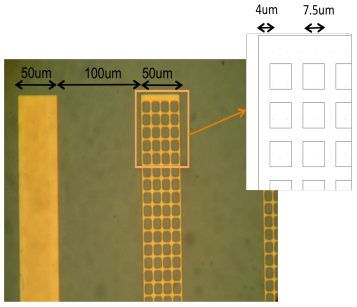

Figure 4: Mesh electrodes, showing dimensions of the mesh structure.

In order to analyze the mesh electrodes, we used the quasi-static application mode in the AC/DC module of Comsol Multiphysics 3.4 and studied the field distribution and impedance for both the types of electrodes. For optimization of electrode geometries, the impedance and capacitance were calculated. Values of impedance (Z) and conductance (Y) were obtained from Comsol, from which resistance (R) and capacitance (C) were calculated by:

$R = Re\ [Z]$ (1)

$C = Im\ [Y]/\omega$ (2)

where ω is the angular frequency. The dimensions of the electrodes were arrived upon by optimizing the R and C values.

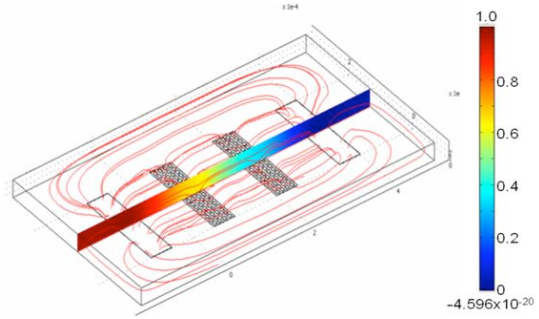

Figure 5: Field lines (streamlines) and potential profile (Cross-sectional slice) for mesh electrodes.

A potential of 1 V was aplied across the outer electrodes and the potential profile was obtained. Field distortion was very small due to the mesh structure as can be seen from the potential profile (Fig 6) especially at a distance away from the electrode plane where the biosamples interact with electric field.

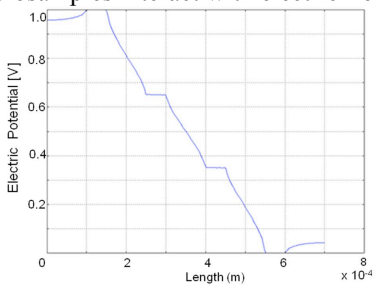

Figure 6: Potential profile across the mesh electrode.

In microfluidics, uniform mixing of reactants before their analysis is essential when multiple solutions are employed. At microscale dimensions, turbulent flow is not possible due to the low Reynolds number so to mix the fluids we used passive methods without the use of any external force [15]. By creating a serpentine channel, turbulence can be created and thus the interfacial surface area between the different fluids can be increased and mixing occurs. We have designed systems with four inlets, typically to permit cell samples, inhibitors, couplers and dyes.

3.3 Ion Sensitive Field Effect Transistors

ISFETs are chemically sensitive solid state electronic devices. They incorporate a MOSFET in which the metal gate is replaced by a chemically sensitive membrane along with a reference electrode. Standard CMOS process was followed in ISFET fabrication (Fig 7). Long source/Drain leads are designed for adequate separation between the pads and the active area exposed to electrolyte [16].

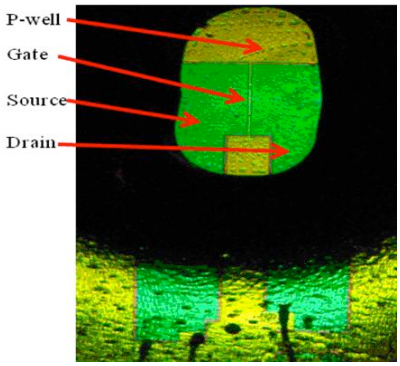

Figure 7: ISFET with gate, source and drain areas shown.

With our BioMEMS lab on a chip, we have incorporated four ISFET sensors viz. pH, Na^+, K^+, Ca^{++}. This pH monitoring is employed in studying physiological conditions of mitochondria and metabolic activities together with selective ions (Na^+, K^+, Ca^{++}) detected by functionalized of ISFETs [17]. In mitochondria, the role of ions gained very strong interest since indentified channelopathies of ions can lead to disorders therefore to diseases and aging; here collapse of the inner membrane potential may occur [18].

4 PRELIMINARY RESULTS

Impedance spectroscopy was done using a Solartron impedance gain/phase analyzer (SR) and the three configurations of electrode for different samples. Fig 8 shows impedance as a function of frequency for water. We can see that the curve for the 2 electrode setup has a much greater slope at low frequencies. The double layer, which is about 10 nm thick for water, causes a larger capacitive

effect, which dominates the impedance at low frequencies. Hence at frequencies below 10 kHz, the impedance curve looks like a capacitor. For the 4 electrode setup, the slope is reduced drastically, and there is further reduction in the electrode polarization effect for the mesh electrode.

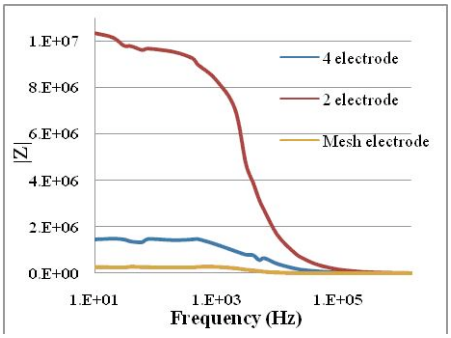

Figure 8: Impedance spectroscopy done on water using different electrode setups.

To study the influence of the mesh electrode on the polarization effects, we performed measurements on an LCR meter (HP). As shown below (Fig.9), we see that as expected, due to smaller contact area while maintaining the overall original size, the mesh electrode contributes less capacitance at low frequencies than the conventional electrodes. It does not completely eliminate the polarization and further corrections are necessary in data analyses so that the obtained results provide reliable information on dielectric parameters.

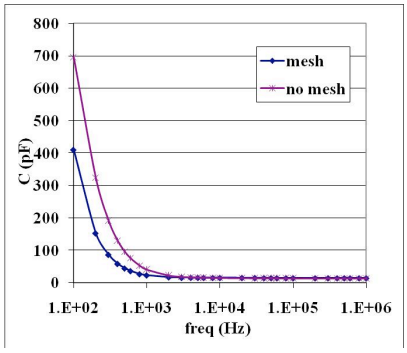

Figure 9: Capacitance – frequency curve for 4 probe setups with mesh and without mesh using water.

Conflicting reports on impedance or capacitance measurements frequently originate from the differences in equivalent circuits representation of the system[19]. Correct equivalent circuits help avoiding misinterpretation of material parameters derived from such measurements, especially at the low frequency regime. That includes nonlinearities of double layer behavior, which is still not fully understood.

In conclusion, we fabricated an integrated BioMEMS device to probe different parameters of the mitochondria vital to understanding the reasons for its dysfunction. The simultaneous measurement of membrane potential, charge and pH would simplify mitochondria disease diagnostics. We can obtain a range of data simultaneously to study mitochondrial bioenergetics better. It will prove useful as a diagnostic tool in studying conditions affected by changes in the mitochondrial membrane potential.

4.1 Acknowledgements

We acknowledge the support from the Texas Coordinating Board ARP grant. W. Z.-W. would like to thank colleagues from The Institute of Electron Technology in Warsaw for their cooperation and support.

5 REFERENCES

[1] A.Trifunovic and N.G. Larsson, J. Internal Med., 263,2, pp. 167-178, 2008.
[2] D.Wallace, Ann. Rev. Genet., 39, pp. 359-407, 2005.
[3] T. Hattori et al., Biophys. J,88, pp. 2340-2349, 2005.
[4] C.Prodan and E.Prodan, J. Phys. D: Appl. Phys. 32, pp.335–343,1999.
[5] W. Franks et al., IEEE Trans. on Bio. Eng., 52,7,pp.1295-1302, July 2005.
[6] W K Wu, C K Liang and J Z Huang, Proc. of the 26th Annual Intl. Conf. of the IEEE EMBS San Francisco, CA, USA, Sept. 1-5,pp 2579, 2004.
[7] G Mattiasson, Cytom. A. 60, p.145, 2004.
[8] K Cheung, S Gawad and Philippe Renaud, Cytometry A 65A, p12, 2005.
[9] L Wang, L Flanagan and A P Lee, J. of Microelectromech. Systems 16, 2, pp.454, 2007.
[10] T. Sun et al., NANO: Brief Reports and Reviews, 3, 1, pp. 53-63, 2008
[11] T. Sun et al., Lab on a Chip, 7, pp. 1034-1040, 2007.
[12] J. H. Miller, Jr., et al, J. de Physique IV (France), 131, pp.363-366, 2005.
[13] Zu-Yao Chang, G A M Pop and G C M Meijer, IEEE Trans. on Bio. Eng', 55, 3, p.1247, 2008.
[14] A R A Rahman, D T Price and S Bhansali, Sens. and Act. B, 127, pp.89-96,2007.
[15] R H Liu, M A Stremler, K V Sharp, M G Olsen, J G Santiago, R J Adrian, H Aref and D J Beebe, J. of Microelectromech. Sys., 9, 2, pp.190,2000.
[16] D. Tomaszewski, C. M. Yang, B. Jaroszewicz, M. Zaborowski, P. Grabiec and D. G. Pijanowska, J. of Telecomm. Inf. Tech., 55-60,3/2007.
[17] M. Lehmann et al., Biosensors &Bioelectronics, 15, pp. 117-124, 2000
[18] Y. Pi et al., J. Mol. Med., 85, pp. 937-951, 2007.
[19] M. Umino et al., Medical and Biolog. Eng. And Computing, 40, pp. 533-541, 2002