Microfluidic biosensor for single cell high speed flow impedance spectroscopy

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Abstract—**This paper presents a micro-biosensor based on Electrical Bio-Impedance Spectroscopy (EBIS), applied to blood cells characterization and diagnosis. This sensor uses microfluidic flow, coupled to EBIS system to perform cell by cell measurements at high flow rate, up to several hundred cells per second. Two devices were realized with different materials, SU_8 resin and polydimethylsyloxane PDMS to structure the microchannel. PDMS device stand higher pressure than SU_8, permitting to improve sensor reliability and the possibility to clean it. Measurements confirm it ability to detect and characterize each cell and particle in less than 1ms during their passage in a microchannel, allowing high speed diagnosis of a large amount of cells. Calibrated particles were used to check the proper functioning of the sensor and the possibility to detect and measure few variation induce by the passage of cells.**

biosensor; microfluidic; impedance spectroscopy; single cell

I. INTRODUCTION

Increasing of MicroElectroMechanical System (MEMS) and lab on chip devices directs current researcher to find new micro-nano applications and to transpose others existing technologies at micro and nanometer scale. This is the case of impedance spectroscopy [1], which allows many prospects for biomedical applications as diagnosis of tissues. This method permits to determine the physiological status of bio-samples by the measurement of electrical response of living tissues and cells. Some pathologies induce changes in physical structure and chemical composition of bio-samples, thus changing electrical impedance, and can be measure and interpret. These types of measurements are performed quite a long time on a large scale of tissues and bio-samples at macroscopic scale [2], and many models have been developed [3]. Macroscopic measurements provide general information about a large amount of different tissues which compose the global sample. One of the best examples concerns blood, composed of many really different particles as White Blood Cells (WBC), Red Blood Cells (RBC) and platelets. WBC just represent less than 1% of particles, and their impact is neglected in whole blood. Naturally, current applications are focused on characterization of microscopic samples to improve sensitivity detection. Interdigitated electrodes are one example of electrodes evolutions for small samples measurements [4][5] and single cell analysis. The most current used approaches for single cells and particles characterization are based on coulter counter method [6]. It permits to detected particles crossing a micro-hole drills in a thin membrane, which separate two tanks with measurement electrodes. Even if this method is easier to realized, it presents a higher critical volume in the sensing area and inducing more unusable measures and very high sample dilution. Another technic consist in capture cells by aspiration on micro-holes [7]. It able to characterized them with high sensitivity but for a few amount of total cells present in the sample. For this, flow cytometry with direct measurement in a microchannel is more and more developed for single particles measurements.

This paper is based on previous works [8], center around on a microfluidic biosensor design for cell by cell measurement in dynamic. It combines microfluidic displacement and impedance spectroscopy to detect and characterize single cells at high flow rate. Microfluidic properties, used in this device, able to direct and focused some kinds of particles, one by one in restricted measurement area composed of microchannel

The second section describes the sensor structure with the different parts of sensing area, composed of microelectrodes. We expose the possibility to characterize different cell properties at high flow rate, during their passage in the sensing area. Theories for electric and dielectric properties of cells suspensions are exposed, as mathematic and electric equivalent models; essential for measurement's interpretations.

Third part is focused on sensor manufacturing using microfabrication technologies. The choice of different materials is detail in order to be as compatible as possible, and interact the least with samples. Two devices, made with different materials were conceived and compare during flow tests.

In the Fourth part, a description of measurement setup is exposed. It is based on lock-in amplifier to performed high speed impedance measurements at different frequencies. Measurements realized with cells and particles prove the ability to measure each cell in less than 1ms. Finally, section 5 concludes about the validity of single cell measurement and the respect of stated assumptions.

II. THEORETICAL

A. General structure

This microfluidic sensor is composed of 2 inlet and outlet tanks for liquid sample deposition, connected to a 1000µm long, 20µm wide, and 10µm high microfluidic channel, as presented in Fig. 1. These dimensions are in the same order than a large amount of cells and bacteria as blood cells or yeast cells, commonly used as references. Their choice was already discussed in our previous works, and offer a good compromise between manufacturing difficulties and sensitivity. It contains measurement area formed with two pair of microelectrodes, allowing the ability to perform simple and differential measurements and monitoring particles speed.

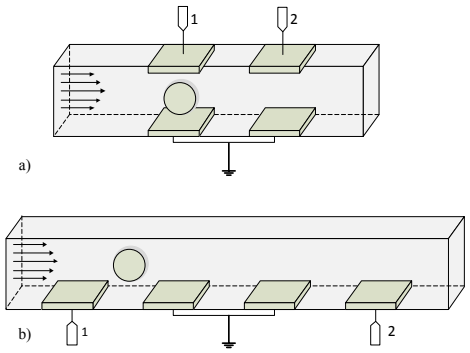

Figure 1. Design of the measurement area for parallel micro-electrodes a) and coplanar microelectrodes).

B. Modeling

Fluidic displacement between tanks and central channel is realized with successive narrowing to create a funnel effect and centering particles. Majority of microfluidic channels provide a laminar flow, wealthy lot of interesting properties. One of them is the attribute to produce a large speed gradient difference between the center (high speed) and the edges. This particularity is effectively used to center cells and decrease the risk of aggregates formation.Modeling

Typical impedance of biological samples is characterized by a high impedance in low frequencies, which decreases stage by stage while keeping a zero or negative phase, proving their electric and dielectric comportments. According to this, sample impedance measurements are often represented by their conductivity and permittivity spectra, where each change of level corresponds to a relaxation: conductivity increase each time while permittivity decrease. Typically, a tissue presents 3 relaxations α, β and γ as shows in Fig. 2. The first is due to polarization of cells membranes, the second to capacitive effect of membranes and the last to dipolar orientation of water molecules. A characteristic frequency can be define for each one, and respectively operate in the range of Hz to several Hz, several kHz to MHz and higher than 1GHz.

Impedance measurement in flow spectroscopy is mainly focused on β relaxation. At these frequencies, impedance measurements can be realized in just several µs, and allow prospect to characterized samples at high flow rates. The γ relaxation operates at RF frequencies and depends of the properties of water molecules. At low frequencies, the high impedance is also due to the double layer impedance. It relates to complex electrochemical reactions appearing in a very small thin layer in the interface between electrodes and sample during electric polarization [9]. It measurement can provide interesting information about the chemical species present in an electrolyte as plasma, but not about isolated cells. In flow spectroscopy, it not represents a useful measure because just depends of medium and is totally independent of cell's properties. Unlike, this effect restrict the evaluation of intrinsic characteristics of cells in low frequencies and can be modeling by a capacitance in series with the sample impedance.

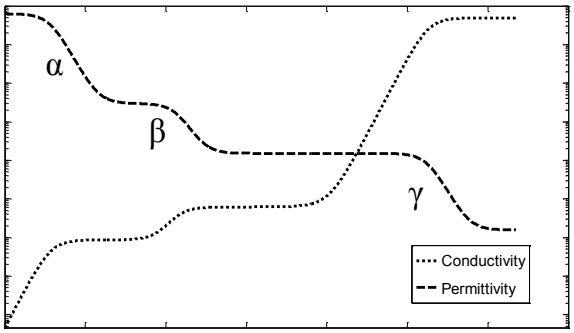

Figure 2. Characteristic Electric and dielectric spectrum of a living tissue.

Biological samples, and especially single cells can be modeled using Maxwell Mixture Theory (MMT), a mathematical model, or Fricke's electrical model [10], as presented in Figure 3.

Each cell component is symbolized in this schematic by it equivalent conductance and capacitance, image of their electrical and dielectric properties. C_{med} and R_{med} respectively represent dielectric and electric properties of medium, R_i electric properties of cytoplasm, C_{mem} the dielectric properties of cell membrane and Z_{dl} the double layer impedance. To equate them for a single cell suspension, Hywell Morgan [11] propose optimizations and simplifications of MMT. It was define at the beginning for a mixture with a large amount of different particles, and not for just one. Calculation of each parameters is possible using (1) to (4). σ_{med} and σ_i are the conductivities of medium and cytoplasm, ε_{med} the electric permittivity of medium, $C_{mem,s}$ the membrane capacitance per surface units, Φ the volume ratio between cell and measurement area, and k a cell factor just depending of electrodes geometry.

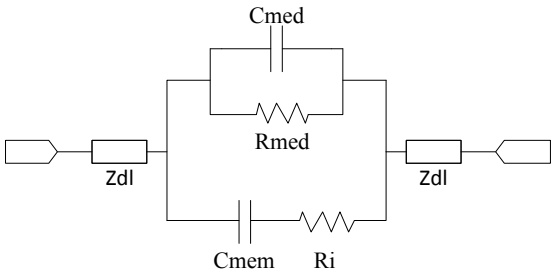

Figure 3. Electric Schematic of Fricke cells suspension model.

$$R_{med} = \frac{1}{\sigma_{med}(1-3\phi/2)k}. \quad (1)$$

$$C_{mem} = \frac{9\phi r Cmem,s}{4}k. \quad (2)$$

$$R_i = \frac{4\left(\frac{1}{2\sigma_{med}}+\frac{1}{\sigma_i}\right)}{9\phi k}. \quad (3)$$

$$C_{med} = \varepsilon_{med}(1-3\phi/2)k \quad (4)$$

III. SENSOR MANUFACTURING

This section describe the fabrication of devices with coplanar electrodes of 20x20µm² and spaced by 20 µm, in a 10 µm high and 20µm wide microchannel. These dimensions and structures were choice following simulations results obtained in previous works. Decreasing the gap between coplanar electrodes reduces the uniformity of electric field in the measurement area, and concentrate it at the bottom of channel. On the other hand, increasing this gap reduce the ratio Φ, and thus, the sensibility of sensor.

A. Materials

In biomedical applications, materials choice is very important in order to improve measurement sensitivity and avoid samples contaminations. As we shown in the previous part, electrodes polarization is the root of complex electrochemical reactions. These act as barrier for low frequencies measurement, and can modify sample properties by salting out some chemical species. It is possible to strongly reduce this using materials as biocompatible as possible. To monitor the evolution of particles in the microchannel during experimentation, materials used to make it should be transparent to visible light, to be able to use optic microscope. The last exigency concern the resistance of each material against chemical agents as acetone or ethanol. Biological samples are very hard to completely remove after passage in microfluidic device, particularly because of capillarity effects. In this cases, only hard cleaning can be efficient to set a sensor reusable. Our choice was carried on the following materials:

- Glass (substrate): Biocompatible, relatively inert for many materials and completely transparent to visible light.
- Platinum (electrodes): Noble metal, one the most biocompatible.
- SU_8 resin: One of the most biocompatible and used in microfluidic applications, ability to create high ratio patterns and transparent.
- Polydimethylsiloxane (PDMS): One of the most biocompatible and used polymer in microfluidic applications, can be mold with sub-micrometric patterns and transparent.

B. Manufacturing

According to the dimensions of useful components of this system, UV lithography is particularly well suited. Two different methods were used to make this device. The first consists to structure the channel in SU_8 resin and enclosed it with a PDMS cover. The second consists to directly structure the channel in a PDMS block by molding. Both methods using the same technics to realize platinum electrodes, by deposition of a 150nm layer on all glass subtract by sputtering. Patterning was performed by structuration of S1813 photoresist and dry etching by plasma, as demonstrated on Fig. 4.

For the first sensor, a 10µm SU_8 layer were deposit and structure by UV lithography to form the channel. This one were irreversibly enclosed using 3mm flat PDMS block. Inlet and outlet tanks were previously pushed through PDMS. For the second, a mold were previously made by structuration of AZ9260 resin with a negative lithography mask. Microchannel was directly mold on PDMS block and irreversibly glued to glass substrate after 0_2 plasma exposure. Figure 5 expose the sensor structure finally obtained.

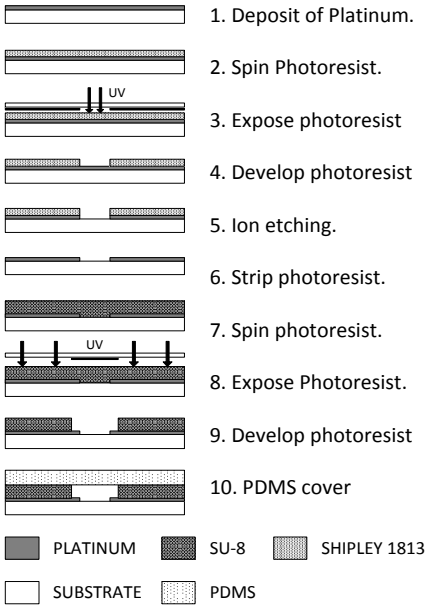

Figure 4. Schematic of the main steps of lithography

C. Microfluidic tests

Before to perform any measurements, our design were preliminary tested in flow conditions to check correct fluidic displacement and the ability to clean it after used. For that, blue ink was injected using syringe, as display in Fig.5. In both cases, we obtained laminar flow without any leak in normal conditions (low pressure). A higher pressure around 1 bar, uses to clean or unblock channel, cause SU_8 unsticking from substrate. For the second device, directly made in PDMS, it do not suffer of any damages, and can be unlock and clean at high pressure, as demonstrate on Fig. 6.

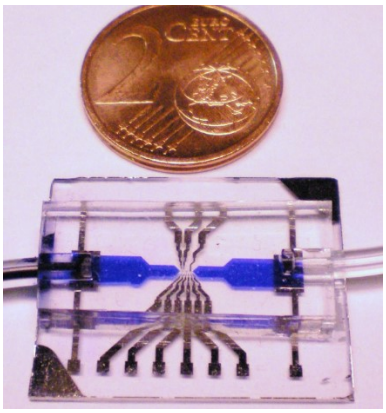

Figure 5. Photograph of the microfluidic device

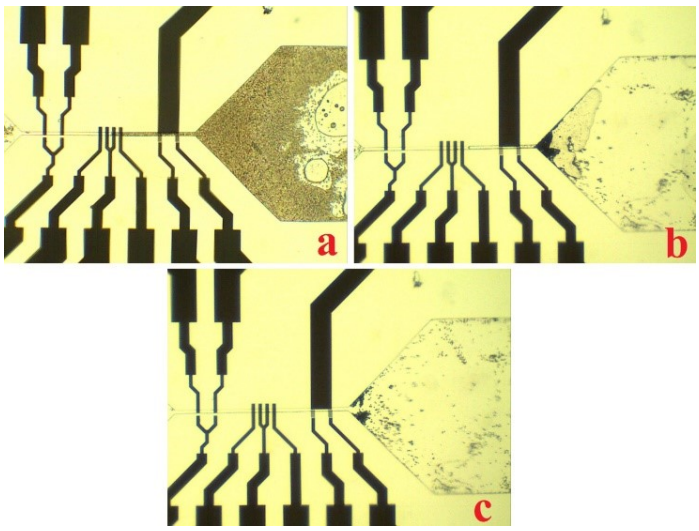

Figure 6. Unblocking a microfluidic channel blocked by a large quantity of cells: step a to b.

Tests were performed using a very high yeast cells concentration in water, to improve the probability to block microchannel. We obtain it in the first step with an aggregate of thousands cells (Fig.6.a). After 1 minute of acetone injection at pressure around 1 bar, the majority of cells were dissociated and evacuated through channel (Fig.6.b). One other minute after, the inlet were unblocking, allowing again fluidic displacement (Fig.6.c). Even after acetone cleaning, some cells stay blocked due to high contact interactions in micrometric scale, but can be completely removed using both fluidic pressure and ultrasounds cleaner.

IV. Experimentation and Results

A. Measurement setup

Flow impedance measurement require dedicated measure chain, able to perform high speed measurements. Cells and particles should be detected and measured during their passage, without immobilization, in sensing area. This one is very short, just few µm, and cell flow is difficult to control and regulate. Moreover, it is necessary to use different frequencies signals in order to completely characterize it. Classical impedancemeters and network analyzers, based on frequencies sweeping, cannot be used there. The only method available is to send a multiplexed signal including all needed frequencies. For that purpose, the impedance spectrometer HF2IS of Zurich Instrument, based on lock-in amplifier principle, were choice to perform our measurements. It permits to measure up to 8 discrete frequencies in a large band, 1µHz to 50MHz, around the β relaxation. Optic microscope with CMOS camera is used to monitor cells displacement during measurement, to check if impedance variations really correspond to cell detections and avoid the risks of channel blocking. A global schematic is show on Fig. 7.

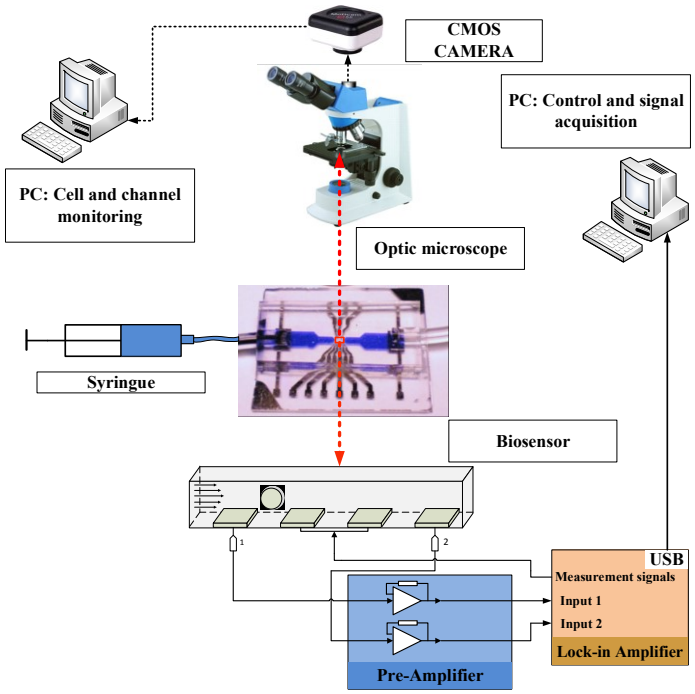

Figure 7. Measurement setup used for monitor and characterized

B. Results

First experiments were realized with simple bacteria, yeast cells, diluted in physiological salt solution. Measurement were performed with a single frequency at 500 kHz, in the band between α and β relaxation. At this frequency, interface impedance can be neglected and the cell considerate as an insulating material. Measured impedance is just a function of medium resistance, and cell dimension. Impedance variation during the passage of a particle in area measurement is image of particle's size. Figure 8 show this resistance variation during the passage of two bacteria of different sizes.

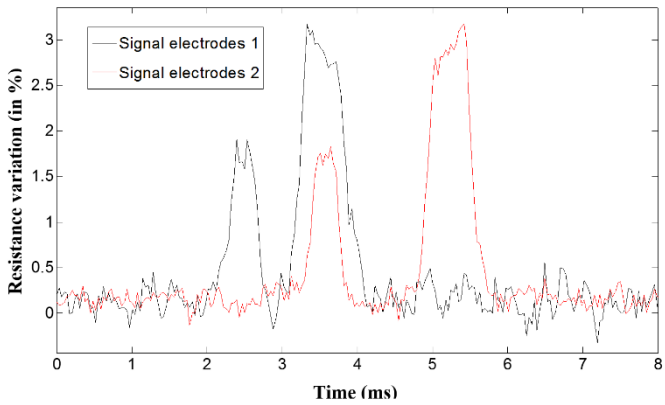

Figure 8. Resistance variation measured during the passage of two different cells

As variations induced by the passage of cells are proportional to cell's sizes, we can prove the first particle is smaller than the second. The two pairs of electrodes measure the same variations, in order of 2 to 3 %, proving the ability of our system to measure small variations with good precision. Time detection is less than 1ms per microparticles, permitting to measure up to several hundred particles per second, in agreement with our assumptions.

Second experimentations were accomplished using 6 µm polystyrene calibrated microbeads. Even if previous resistance variations can be used to compare particles sizes, we need calibrated particles to check if our measured variations agree with theory. For that, beads were diluted in the same buffer and measured in the same conditions than bacteria. For each detected bead, resistance variation was recorded. All results was compiled in a normalized repartition graphic giving in Fig. 9. The theoretical repartition was calculated using bead's datasheet and theoretical formulas, and represent the repartition guaranty by the manufacturer. For this reason, this repartition is always superior or equal to the reality. Our measurement are perfectly in concordance with the predictive variation, with a well centered repartition.

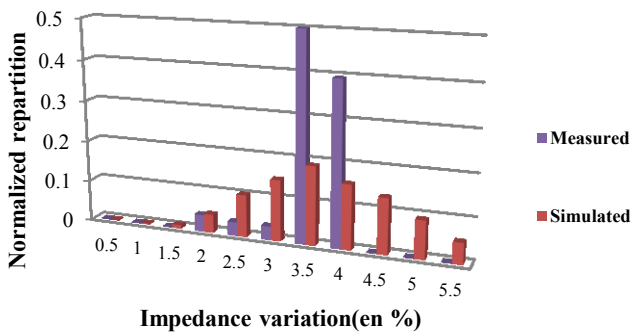

Figure 9. Repartition of calibrated microbeads. Results from experimentation and simulation

V. CONCLUSION

A sensor based on impedance spectroscopy and microfluidic device, able to perform dynamic cells measurements was described and characterized. Two devices were manufactured using SU_8 resin and PDMS silicon polymer. Even if both were able to perform flow impedance spectroscopy, the device just using PDMS allows better performances and the possibility to efficiently clean it after multiple tests.

Results obtained prove the ability of our device to detect and characterized particles and cells in less than 1ms, allowing the possibility to analyses up to several hundred cells per second. Repartition measured with beads show our system permits to detect very few impedance variations with a good precision and repetability.

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