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Discrimination of Glioblastoma Cancer Stem Cells by measuring their UHF-Dielectrophoresis Crossover Frequency

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***Abstract*—This paper introduces firsts results of characterization of glioblastoma cell lines; measuring their crossover frequencies by dielectrophoresis (DEP) technics in the UHF frequency range (above 50 MHz). LN18 line cells were cultured following different conditions, in order to achieve an enrichment of cancer stem cells (CSCs). The DEP electrokinetic method is used to discriminate the CSCs from the differentiated cells. In this study, microfluidic lab-on-chip systems implemented on Bipolar-Complementary Oxide Semiconductor (BiCMOS) technology is used allowing single cell handling and analysis. Based on measurements of their own intracellular specificities, the enriched CSCs population, cultured in dedicated defined medium, have shown clear differences of DEP crossover frequency signatures compared to differentiated cells cultured in normal medium. That demonstrates the concept and validates the technique efficiency for CSC discrimination in glioblastoma pathology.**

I. Introduction

Gliomas represent the majority of primary brain tumors. Malignant gliomas, including glioblastoma multiforme (GBM) (World Health Organization (WHO) grade IV) are among the most devastating and lethal malignancies, due to the recurrence of tumor, which is resistant to, conventional chemotherapies and/or ionizing radiation. One of the most obvious mechanisms of these treatments escaping is the presence of restricted cell population inside the tumor named cancer stem cells (CSCs). CSCs are able to induce tumor neoformation and progression. Currently, characterization of the CSC populations is based on targeting a set of biological markers. These markers are efficiently used to validate the stemness lineament of the cancer cells from the completely heterogenic tumor cell population. Besides biological properties, physical characteristics of CSCs are expected to be a potential way to discriminate and sort CSC populations. To help medical researchers and scientists to diagnose and prognosis diseases, many different techniques have been implemented [1]. Bioelectric signals from cells have been proven to carry various useful information about the cell status [2]. Many sources of cell bioelectric signals like sodium potassium pumps in the membrane cell matrix or cell-to-cell interactions with the extracellular matrix can be determined by exploiting the dielectric properties. Among these techniques, Dielectrophoresis (DEP) is a label-free, accurate, fast, and low-cost diagnostic technique that uses the principles of polarization and the motion of bio-particles in applied electric fields [3]. The efficiency of this technique has been proved in various fields, including medical research, biosensors, microfluidics and diagnostics. In particular, manipulation of microscopic sized particles, such as trapping or cell sorting, including healthy or cancerous cells suspended in fluid media, has been successfully demonstrated in a variety of ways using DEP methods.

Actually, this technique can also help to sort low represented cell subpopulations, such as cancer stem cells (CSCs), hidden within heterogeneous other differentiated cells. Hence, we show here a new approach for CSCs detection and characterization based on microwave dielectric spectroscopy. Such approach offers unique capabilities to investigate the intracellular dielectric properties and allows screening of the biological differences and properties among the heterogeneity of a tumor.

II. DEP crossover frequency basics

DEP induces motion of particles submitted to a non-uniform electric field when the particles and surrounding medium have different polarizabilities [4]. In the present case, regarding the used sensor electrode geometry [5] (Fig.1), considering a negligible effect of field induced phase changes [6] and cell as a homogeneous spherical dielectric particle [7], the induced DEP force can be then computed using equation (1).

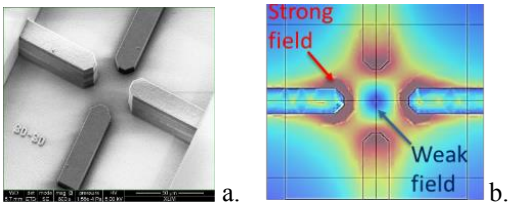
$$F_{DEP} = 2\pi\varepsilon_m r^3 Re[K(\omega)]\,\nabla\,|E_{rms}|^2 \quad (1)$$

$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) \quad (2)$$

Where r is the particle radius, ω is the angular frequency of the applied electric field $E(\omega, t)$, $K(\omega)$ the Claussius-Mossotti given by (2) in which ε_p^* and ε_m^* refer to the complex permittivity of the particle and the suspension medium, respectively, E_{rms} is the root mean square value of the electric field and $Re[K(\omega)]$ the real part of Claussius-Mossotti factor.

By changing the frequency of the applied electric field [8], the polarized particles would array in various motions; determined in fact by the sign of $Re[K(\omega)]$ and which relies on the

difference of polarizability between the particle and its surrounding medium [9].



a. b.

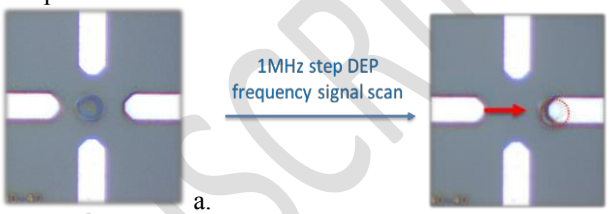
Fig.1. Quadrupole microelectrode sensors implemented in BiCMOS technology (a), electric field gradient on the quadrupole electrode structure (b).

Actually, the generated force is attractive when $Re[K(\omega)]$ is positive and the particle moves toward the electrodes (positive DEP) whereas when $Re[K(\omega)]$ is negative, the particle is repealed away since the force is repulsive (negative DEP). When the force becomes null and the cell switches to negative to positive DEP (or vice-versa), the DEP crossover frequency is then reached. This frequency is thus characteristic of cell own properties and specificities and may differ between different cells.

Depending on the type of cell properties one wants to access, the choice of the DEP frequency range is important. If information about cell plasma membrane specificities are looking for, conventional DEP frequencies (typically from 100 kHz to 5 MHz) are much suitable for the cell analysis. Whereas, UHF-DEP frequencies (from 50 MHz to 500 MHz) will be better to provide information about intracellular properties. Indeed, when frequency increases above several tens of MHz, the plasma membrane lets the electric field penetrate the cell and interact directly with the cell interior. As a result, the effect of DEP forces generated at high frequency regimes may be different according to the own cell content dielectric specificities (i.e. attract vs. repeal). As a result, since stem cells versus differentiated ones exhibit different biological properties or physiological mechanisms linked to their nature, origin, differentiation state, pathological state, aggressiveness level…, analysis of their dielectrophoresis behavior under UHF frequencies seems very relevant for the targeted application.

III. Tools and Methodology for Cell Crossover Frequency Measurement

The main purpose of this study is to characterize LN18 cell lines to identify their DEP crossover frequencies and establish DEP signature according to their different culture conditions (normal culture medium *vs.* defined medium). Each cell population is introduced into the sensor suspended in a DEP medium (water/sucrose – pH: 7.4; conductivity: 26 mS/m; osmolarity: 280 mOsm) by a fluidic inlet driven by a flow controller (Fluigent MFCS) and flows in a PDMS (Polydiméthylsiloxane) microfluidic channel implemented above a microelectrode system. The experiments were done using a 40x40µm gap quadrupole electrodes design (Fig.1.) [10]. A frequency adjustable DEP signal has been applied to the left and right electrodes whereas top and bottom ones were grounded. Once electrodes were biased with a 500 MHz DEP signal, single cell was trapped (Fig.2.a) in the center of quadrupole electrodes sensor by negative DEP and the flow was stabilized (reaching an inlet and outlet pressure equilibrium at each microchannel end). DEP signal was first turned off to well stabilize the trapped cell in the center, between the electrodes, as an evidence that the investigated cell is no longer subject to other motion forces. Finally, gradual frequencies decreases were applied in order to determine the cell crossover frequency: occurring at the moment when the cell started to be attracted by one lateral electrode (positive DEP). At the end, the cell was released and a new cell was trapped and characterized following the same principle.



a. b.

Fig.2. Cell trapping– repulsion at system center - DEP < 0 (a), Crossover frequency identification before cell attraction to electrode DEP > 0 (b).

IV. Cell Preparation

Experiments were performed on LN18 cell line derived from malignant gliomas from adult patients, purchased from the ATCC. Cell pools were cultured at 37˚C in a humidified 5% CO2-95% air incubator in two conditions: (i) normal growing conditions in DMEM plus Glutamax complete medium (called NM for "Normal Medium") (ii) stringent conditions in selective DMEM/F12 medium (Defined Medium (DM) where main grow factors are removed).

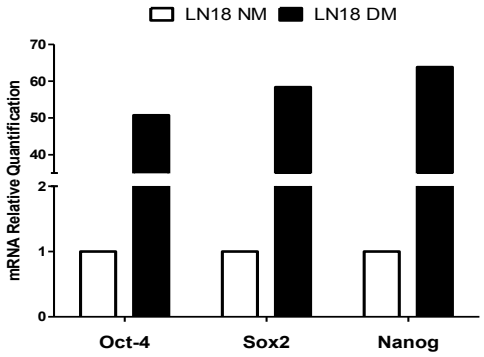


Fig. 3. Comparative analysis of gene expression of Oct-4, Sox2 and Nanog, three CSCs markers, in LN18 cell line, cultured in normal medium (NM: white histograms) or in define medium (DM: black histograms), measured by Quantitative PCR (Polymerase Chain Reaction).

Actually, with such stringent conditions, mainly highest resistance cells with strong aggressiveness special features survive and can grow. First, control experiments were assessed to confirm the enrichment of CSCs in defined medium. Comparative analysis of the gene expression (mRNA levels) of the stemness lineament was assessed in the cells cultured 6 days in normal culture medium *vs.* in defined medium (Fig.3). Analyzed CSCs biological markers showed an overexpression in cells

cultured in define medium compared to those cultured in normal culture medium. These results confirm the enhancement of the expression of the stem cell phenotype.

V. Characterization of LN18 DEP crossover frequency

Fig.4 represent examples of LN18 cell trapping and frequency characterization of each culture conditions (normal and defined medium respectively). For the statistical study, the chosen crossover frequency corresponds to the frequency for which the trapped cell starts to move away the electrodes quadrupole center (positive DEP beginning): i.e. respectively 122MHz and 65MHz for Fig 4.a & 4.b cases.

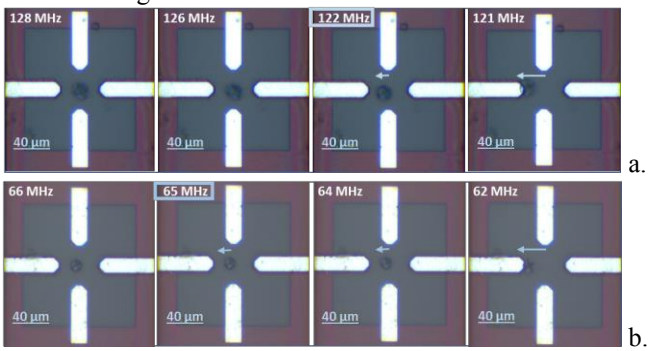


Fig. 4. Microscope imaging sequence of LN18 cell crossover frequency measurement by tuning the DEP signal frequency - Cultured in NM (122 MHz) (a), Cultured in DM (65 MHz) (b).

The CSCs enriched population shows lower crossover frequencies comparing to the cells cultured normal conditions (Fig.5) – Average of 132 MHz for NM vs. 64 MHz for DM - This decrease demonstrates a significant difference between these two population profiles, proving a real difference on the dielectric characteristics of the CSCs compared to differentiated cells. This difference raises up a great potential of cell sorting of these two populations, allowing a discrimination of cell subpopulations forming tumor mass.

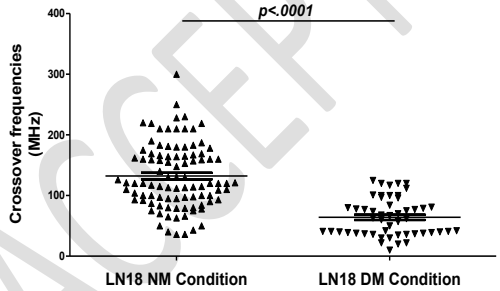


Fig.5. Significant differences of crossover frequencies in LN18 cells, cultured in DM & NM conditions. The p value was determined using t test.

The set of statistics concerning the characterization of LN18 cell crossover frequencies is summarized in table 1. As shown a significant number of cells have been characterized showing statistically consolidated data. The larger standard deviation observed for LN18 NM cell pool can be explained by the natural cell line heterogeneity: including a large number of different differentiated cells but also some few CSCs occurrence in the pool. Whereas the LN18 DM cell pool may concentrate a much higher number of un-/low differentiated and under differentiation cells and much numerous CSCs too; since DM culture conditions are not favorable for differentiated cell grown.

TABLE I- Summary of crossover frequency measurement (MHz)

Cells lines	Number of cells	Avg	Median	Dev Std	Error Std	Min	Max
LN18 NM	95	132	120	53,36	5,47	36	300
LN18 DM	51	64	62	31,22	4,37	10	125

VI. Conclusion

We demonstrated here a new approach for CSCs real time discrimination using microfluidic lab-on-chip (LOC) platform implemented on CMOS technology. CSCs detection and characterization, based on microwave dielectric spectroscopy, offers unique capabilities to investigate the intracellular dielectric properties. Differences observed in the crossover frequencies of each subpopulation, showed a great potential for the development of a novel method to characterize and to discriminate cancer stem cells. This technique allows screening of the intracellular differences of physical properties of the cell subpopulations. These results are correlated to the biological differences at the functional level reflecting the intracellular changes in CSCs, providing their high aggressiveness potential. Finally, this method confirms a high potential of the lab-on-chip (LOC) platform in the diagnosis and the cure of glioblastoma.

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