UHF-Dielectrophoresis Crossover Frequency as a New Marker for Discrimination of Glioblastoma Undifferentiated Cells

R. Manczak, S. Saada, T. Provent, C. Dalmay, B. Bessette, G. Begaud, S. Battu, P. Blondy, M.O. Jauberteau, C. Baristiran Kaynak, M. Kaynak, C. Palego, F. Lalloue, A. Pothier

Abstract **-** This article introduces the first results of dielectric spectroscopy characterization of glioblastoma cells; measuring their crossover frequencies in the Ultra High Frequency range (above 50 MHz) by dielectrophoresis techniques. Experiments were performed on two glioblastoma lines: U87-MG and LN18 that were cultured following different conditions, in order to achieve different phenotypic profiles. We demonstrate here that the presented dielectrophoresis electrokinetic method can be used to discriminate the undifferentiated from the differentiated cells. In this study, microfluidic lab-on-chip systems implemented on Bipolar-Complementary Oxide Semiconductor (BiCMOS) technology are used allowing single cell handling and analysis. Based on characterizations of their own intracellular features, both selected glioblastoma cell lines cultured in distinct culture conditions have shown clear differences of DEP crossover frequency signatures compared to differentiated cells cultured in normal medium. These results support the concept and validate the efficiency for cell characterization in glioblastoma pathology.

Keywords **— BiCMOS Chip, Biological cell manipulation, Glioblastoma cells, High Frequency Dielectrophoresis.**

I. Introduction

Glioblastoma (GBM) is one of the most frequent and the most aggressive tumor of the central nervous system. About 240,000 brain tumor new cases were diagnosed worldwide; the majority are GBMs with an incidence of 3–4 per 100 000 persons per year [1]. Conventional therapeutic strategy is mainly surgery, in combination with chemo- and radiotherapy according to Stupp protocol. Despite recent advances in surgery, imaging, radiation therapies and chemotherapy, the median survival is less than 15 months [2]. This dark prognosis of GBM is primarily due to the recurrence of tumor, which is resistant to pre-cited conventional treatments [1].

Limited advances in glioblastoma treatment are closely linked to the existence of a restricted cell subpopulation also called cancer stem cells (CSCs), some very immature and undifferentiated cells, responsible for tumor cell heterogeneity [3]. However, even genetically diverse clones express undifferentiated cell markers related to cancer stem cells such as CD133 and CD44. The higher expression levels of CD133 have been correlated to poorer prognosis suggesting that this marker might play a significant role in the resistance of this type of cancer to chemotherapy and radiotherapy [4]. Other markers such as the transcription factors OCT-4, SOX2, pSTAT3 and NANOG are considered as key players in regulating transcription of glioblastoma CSCs [3]. These CSCs are a subpopulation of undifferentiated cells, which have specific biological properties similar to normal stem cells. Currently, biologists use some immunostaining approaches to characterize CSC populations, as flow cytometry, optical microscopy or protein array analysis, based on targeting a set of undifferentiated markers previously described. These markers are required to validate the stemness lineage of CSCs from the huge heterogeneous cell population. Nevertheless, CSCs subpopulation are very rare in tumors and their isolation often requires enriching them in specific culture medium. This strategy is time consuming and delays the results. Currently, the key objective is to try to get around this problem by establishing a new way to discriminate and sort undifferentiated cell populations specifically according to theirs biological and physical characteristics.

To optimize the diagnosis and prognosis methods, the development of different approaches and techniques based

This work was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No 737164.

R. Manczak (e-mail: remi.manczak@xlim.fr), T. Provent (e-mail: thomas.provent@xlim.fr), C. Dalmay (e-mail: claire.dalmay@xlim.fr), P. Blondy (e-mail: pierre.blondy@xlim.fr) and A. Pothier (e-mail: arnaud.pothier@xlim.fr) are with XLIM-UMR 7252, University of Limoges/CNRS, 87060 Limoges, France.

S. Saada (e-mail: sofiane.saada@unilim.fr), B. Bessette (e-mail: barbara.bessette@unilim.fr), G. Begaud (e-mail: gaelle.begaud@unilim.fr) , S. Battu (e-mail: serge.battu@unilim.fr), M.O. Jauberteau (e-mail: m-o.jauberteau-marchan@unilim.fr) and F. Lalloué (e-mail: fabrice.lalloue@unilim.fr) are with CAPTuR-EA 3842, University of Limoges, 87025 Limoges, France.

C. Baristiran Kaynak (e-mail: baristiran@ihp-microelectronics.com) and M. Kaynak (e-mail: kaynak@ihp-microelectronics.com) are with IHP, 15236 Frankfurt (Oder), Germany.

C. Palego (e-mail: c.palego@bangor.ac.uk) is with the Bangor University, Bangor, LL57 1UT, United Kingdom.

on bioelectric signals of cells have been proved to carry various helpful information on cell status [5]. Many sources of cell bioelectric signals, like sodium potassium channels and pumps in the plasma membrane, may affect chemical analytes homeostasis, cell patterning and cell-to-cell interactions with the extracellular matrix, which can be determined by exploiting the dielectric properties. Among these techniques, Dielectrophoresis (DEP) is a label-free, accurate, fast, and low-cost analysis method that uses the principles of polarization and the motion of bio-particles in applied electric fields [6]. The efficiency of this technique has been proved in various environmental and medical fields, including polymer research, biosensors, microfluidics and diagnosis based on microfluidic biosensors [7]. In particular, manipulation of microscopic sized particles, such as trapping or cell sorting, including healthy or tumor cells suspended in microfluidic media, has been successfully demonstrated in a variety of ways using DEP methods [8]. Regarding the cellular heterogeneity in a tumor mass and different cellular subpopulations functions, we can applie the principle of DEP separation method to the cell mixture composing a tumor, especially to discriminate two cellular subpopulations with opposite differentiation properties. Based on this principle, highly represented population composed by differentiated cells are singled out from a lower undifferentiated subpopulation, with stemness properties. Hence, we show here a new approach to detect and characterize the undifferentiated cells subpopulation based on microwave dielectric spectroscopy in the Ultra High Frequency range (UHF), using DEP cell electromanipulation.

This approach offers unique capabilities to investigate the differences on the intracellular dielectric properties of each cell population [9-12] and allows screening of the intracellular biological properties and differences within the heterogeneity of a tumor.

II. Basics on Cells Electromanipulation by DEP

When particles presenting different polarizabilities than the surrounding medium in which they are suspended are submitted to a non-uniform electric field, a DEP force is generated inducing motion of particles [13]. There are different ways to exploit this phenomenon. In the present case, a quadrupole microelectrode system [14] has been used as sensor and implemented in a microfluidic channel (Fig.1). For such electrode geometry, the DEP theory [7, 15, 16] shows that considering a cell as a homogeneous spherical dielectric particle, the induced DEP force can be then computed using equation (1).

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

$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) \quad (2) \; ; \quad \varepsilon_x^* = \varepsilon_0\,\varepsilon_x - j\frac{\sigma_x}{\omega} \quad (3)$$

Where r is the particle radius, ω is the angular frequency of the applied electric field, E_{rms} is the root mean square value of this electric field, ∇ is the gradient operator and $Re[K(\omega)]$ the real part of Claussius-Mossotti factor $K(\omega)$ given by (2) in which ε_p* and ε_m* refer to the complex permittivity of the particle and the suspension medium, respectively. The complex permittivity ε_x* are defined in (3), where ε_x and σ_x are the relative permittivity and conductivity either of particles or immersion medium, and ε_0 represent the electric constant ($8.854\ 10^{-12}$ F m^{-1}).

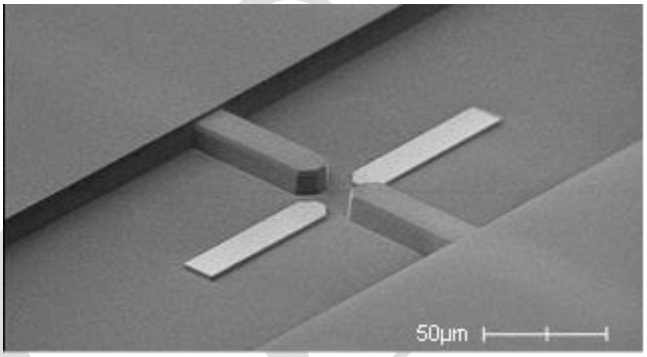


Fig. 1. Quadrupole electrodes system implemented in BiCMOS back end of line SG25H4 technology from IHP.

By changing the frequency of the applied electric field [13], the polarized particles would behave in various ways depending on the magnitude and the sign of $Re[K(\omega)]$ which is in turn determined by the effective conductivity and permittivity of the particle and the dielectric properties of the surrounding medium. Therefore, particles can be individually electro-manipulated according to their own dielectric properties.

Actually, the generated force is repulsive when $Re[K(\omega)]$ is negative, meaning that the particle is repealed away from electrodes (Fig.2.a. negative DEP case - nDEP). Whereas when $Re[K(\omega)]$ is positive, the force is attractive and the particle moves toward the electrodes where the electric field magnitude is high (Fig.2.b. positive DEP case - pDEP). When the force becomes null just before the cell switches to negative to positive DEP (or vice-versa), the DEP crossover frequency is then reached. This frequency can be thus considered as characteristic of a cell own properties and specificities and may differ between different cells.

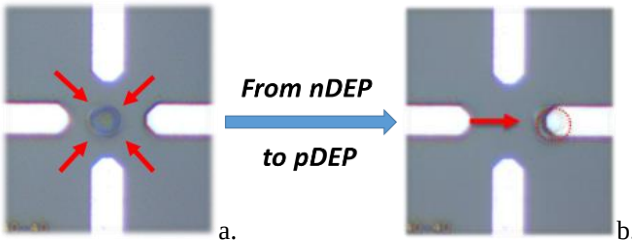


Fig. 2. DEP single cell manipulation principle – Cell Repulsion at system center with nDEP (a), Cell attraction toward electrode with pDEP (b).

Depending on the type of cell properties one wants to

access, the choice of the DEP frequency range is important [17-19]. If information about cell plasma membrane specificities are sought, conventional DEP frequencies (typically from 100 kHz to 5 MHz) are very suitable for cell analysis. At this low frequency range, the cell shape, morphology and size have strong influence on the interaction with the electric field. Conversely, Ultra High Frequencies DEP (from 50 MHz to 500 MHz) will be better to provide information about intracellular properties.

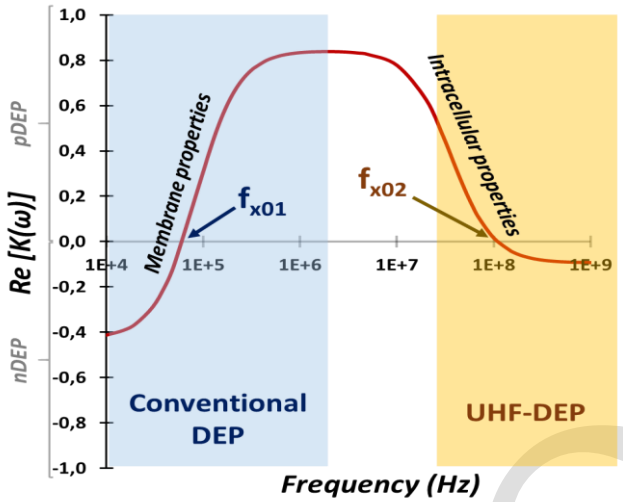


Fig. 3. Typical DEP spectral signature of cell with its two crossover frequencies, f_{xo1} and f_{xo2} respectively at low and high frequency regimes.

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 (i.e. attractive vs. repulsive) at high frequency regimes may be different according to the dielectric properties of the cell content. This UHF-DEP characteristic is often presented in the literature [7, 20, 21] and the crossover frequency f_{xo2} can be written by the equation (4):

$$f_{xo2}^2 = \frac{1}{4\pi^2}\frac{1}{\varepsilon_0^2}\frac{(\sigma_m - \sigma_p)(\sigma_p + 2\sigma_m)}{(\varepsilon_p - \varepsilon_m)(\varepsilon_p + 2\varepsilon_m)} \quad (4)$$

For an aqueous solution, considering $\varepsilon_m > \varepsilon_p$ and $\sigma_p > \sigma_m$, the equation (4) can be simplified with the approximation:

$$f_{xo2} = \frac{\sigma_p}{2\pi}\frac{1}{\varepsilon_0}\sqrt{\frac{1}{(\varepsilon_p - \varepsilon_m)(\varepsilon_p + 2\varepsilon_m)}} \quad (5)$$

The value of the crossover frequency f_{xo2} in the UHF range is directly influenced by the intracellular properties of the cells, largely by its conductivity and to a lesser extent by its permittivity. Specifically, since undifferentiated cells exhibit different biological specificities or physiological mechanisms linked to their differentiation state for example, their crossover frequencies will be different from those of differentiated cells. Therefore, the analysis of their dielectrophoresis behavior under UHF frequencies seems very relevant for the targeted application.

III. Materials and Methods

A. Cellular culture

Two GBM cell lines were tested in this study, U87-MG and LN18. Both of them derived from malignant stage IV gliomas from adult patients, purchased from the American Type Culture Collection (ATCC). Two conditions were used for the analysis: (i) normal differentiation conditions in DMEM plus Glutamax medium supplemented with 10% Fetal Bovine Serum (FBS), 2 mM glutamine and 100 U penicillin/0.1 mg/mL streptomycin, called NN for "Normal Normoxia Medium", (ii) stringent conditions in selective DMEM/F12 medium, Define Normoxia medium (DN), supplemented with 0.6% glucose, 1.2% sodium bicarbonate, 5 mM HEPES , 9.6 µg/mL putrescine, 10 µg/mL ITSS, 0.063 µg/mL progesterone, 2 µg/mL heparin, 20 ng/mL EGF, 20 ng/mL bFGF, 1X penicillin/streptomycin and 2% B27 supplement without vitamin A. Cells are maintained at 37°C, 5% CO2 in a humidified atmosphere - 95% air incubator.

Actually, under stringent culture conditions, mainly only the most resistant cells with strong aggressiveness special features can survive and grow. As illustrated in Fig.4, it is hence expected to achieve a large enrichment in undifferentiated cells in DN cell cultures.

Finally, after two successive centrifuge washes, cells were suspended in an ion free osmotic medium TRIS buffer-based, composed by a water/sucrose mixture with magnesium chloride (pH: 7.4; conductivity: 26 mS/m) conventionally used for DEP experiments. The osmolarity value of this DEP medium, measured with a sample of 70 µL placed in micro-digital osmometer 300-11DR (Type13) varies between 280 and 320 mOSm.

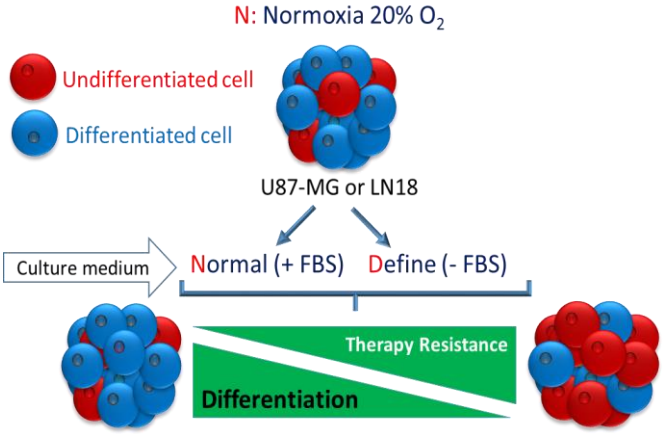


Fig. 4. Representative diagram of the two different culture conditions used for the both GBM cell lines.

B. Tools and methodology for cell crossover frequency measurement

The main purpose of this study is to characterize GBM cell lines to identify their DEP crossover frequencies in the high frequency regime and establish DEP signature according to their different culture conditions (normal culture medium vs. define medium). Each cell population is introduced into the microfluidic chip, suspended in a DEP medium, by a fluidic inlet driven by a flow controller (Fluigent MFCS) and flows in a Polydiméthylsiloxane (PDMS) microfluidic channel implemented above dedicated sensors implemented in BiCMOS technology (Fig.1.). The experiments were done using a 40×40µm gap quadrupole electrodes design. This structure is based on four electrodes, set at 90°, combining a pair of thick (9 µm) electrodes crossing the microfluidic channel with another pair of thin (0.45 µm) electrodes implemented in the middle of the channel [14].

The selected 40µm spacing between each electrodes represent a good compromise between an easy monitoring under microscope of cell motion submitted to both positive or negative DEP forces and the use of moderate RF voltage signal to bias the structure and efficiently act on cells (typically the magnitude of the applied voltage ranges between 2 and 4 V_{pp}). The same frequency adjustable DEP signal has been applied to the left and right electrodes whereas top and bottom ones were grounded. The flow is slowed down, and when a cell arrives near the center of quadrupole electrodes system, the electrodes were biased with a 500 MHz DEP signal expected to be much higher than the f_{xo2} and therefore suitable to efficiently trap cells.

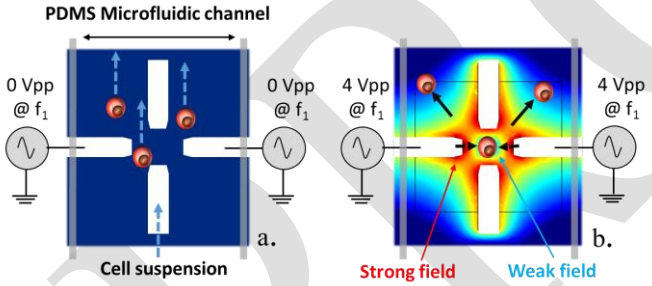


Fig. 5. Cells suspension flowing in the microfluidic system (a), Single cell nDEP trapping at 500 MHz in the center of the quadrupole electrodes related to the generated electric field (b).

At 500 MHz, the cell may react in negative DEP and the generated electric field allows individually catching any single cell present near the system center. Indeed, the strong intensity field areas surrounding the center weaker field zone resulted in an electric field cage where the cell could be efficiently trapped (Fig.5.b.). The others surrounding cells also reacted in negative DEP and were repelled away the analysis area moving to the outside weaker intensity field zones. Then, the flow was progressively stopped and stabilized (reaching an inlet and outlet pressure equilibrium at each microchannel end). Finally, the cell was only submitted to DEP force and natural gravity. The DEP signal was first turned off for few seconds to check that the investigated cell is no longer subject to other motion forces.

Then, it was turned on again setting the signal frequency above the expected crossover frequency and the characterization could start. To determine the investigated cell crossover frequency, gradual frequencies decreases were applied on the DEP signal: first with fast 10 MHz steps and then once approaching the crossover frequency with 1 MHz ones to refine the measurement. Since the crossover frequency occurred just before the moment when the cell started to be attracted by one of lateral electrodes (switching to positive DEP behavior), a slow 1 MHz step frequency scan allowed to accurately observed this moment. Figure 6 illustrates an example of trapping and crossover frequency characterization of a GBM cell cultured in normal medium.

Each cell was characterized hence twice or three times and finally released by increasing the inlet channel pressure to renew medium and trap a new cell for characterization following the same approach.

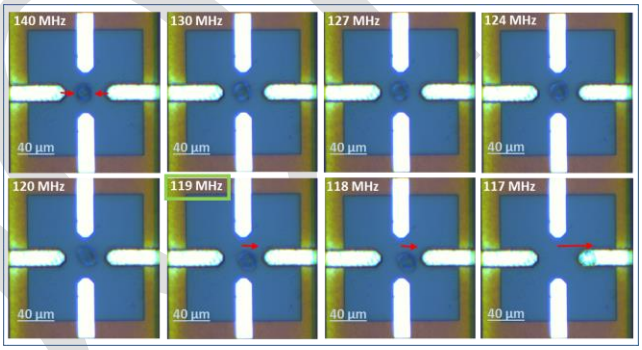


Fig. 6. Cultured in NN - Microscope imaging sequence of GBM cell crossover frequency measurement (119 MHz) by tuning the DEP signal frequency.

This methodology of crossover frequency measurement has been supported and validated though electro-kinetic transient simulations using COMSOL software. Hence, the displacement of the trapped cell, from the center of quadrupole to the edge of the electrodes, according to the decrease of the DEP signals frequency, can be computed as presented in the graph below.

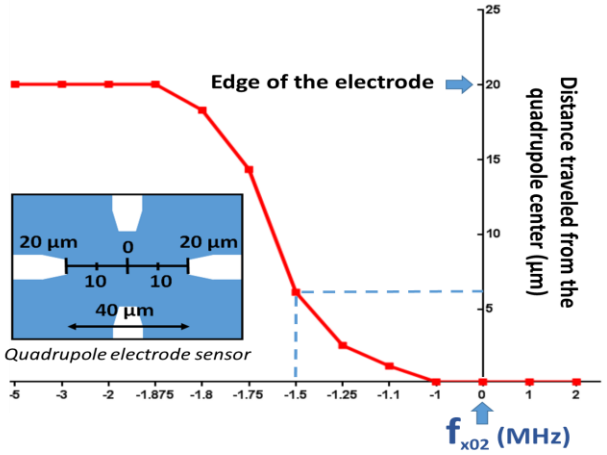


Fig. 7. Simulated cell location change from the center of the quadrupole electrodes system for different DEP signal frequency tuned around the cell crossover frequency.

As Figure 7 shows, the simulations predict that the generated DEP force, once applying a $4V_{pp}$ DEP signal with a frequency set to 1.5 MHz under the cell crossover frequency, should have a sufficient influence to attract and move it 6µm away from its initial negative DEP trapping location. Actually, such displacement value matches well with the ones observed under a microscope during experiments, as illustrated on the Figure 6 photograph taken at 118 MHz. Consequently, we can reasonably consider that using the proposed cell characterization methodology the cell crossover frequency can be measured with a 1-2 MHz accuracy.

IV. RESULTS, ANALYSIS AND CORRELATION

A. *GBM cell lines phenotypic profiles*

First, control experiments were carried out to confirm the enrichment of undifferentiated cell population in define medium. Comparative analysis of the gene expression (mRNA levels) of the stemness lineage was assessed in the cells cultured 6 days in normal culture medium vs. define medium (Fig.8). Analyzed CSCs markers (Oct-4, Sox2 and Nanog) showed an overexpression in cells cultured in define medium (in red) compared to those cultured in normal medium (in blue).

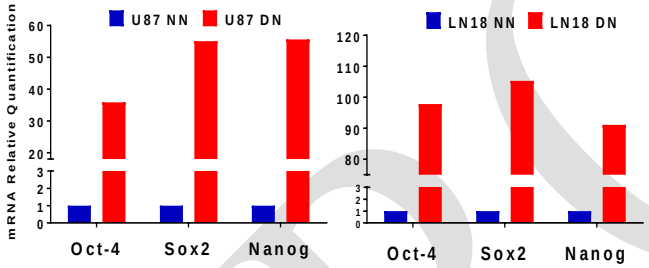
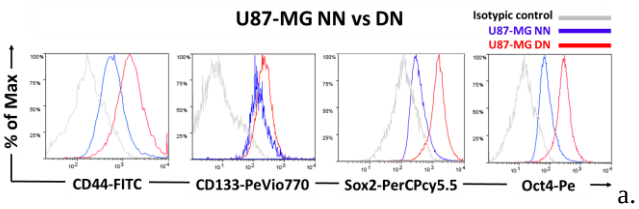
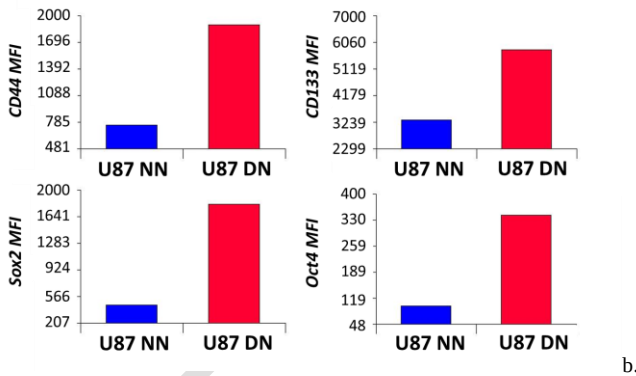


Fig. 8. Comparative analysis of gene expression of three undifferentiated markers: Oct-4, Sox2 and Nanog, in U87-MG and LN18 cell lines, cultured 6 days in normal medium (NN: blue histograms) or in define medium (DN: red histograms), measured by Real Time PCR (Polymerase Chain Reaction).

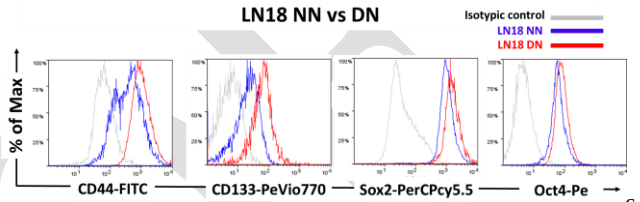
These results were completed by analyzing protein levels with flow cytometry. They showed an enhancement of undifferentiated markers expression in both U87-MG (Fig.9.a. and b.) and LN18 (Fig.9.c. and d.) cell lines cultured in define medium compared to normal medium culture. These biological results validate the enrichment of CSC by culture in define medium.



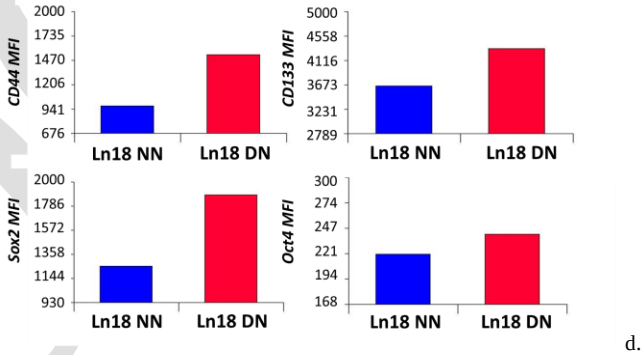
a.



b.



c.



d.

Fig. 9. Comparative analysis of the undifferentiation markers expression CD44, CD133, Oct-4, Sox2 in both U87-MG and Ln18 cell lines, cultured 6 days in normal medium (NN: blue histograms) or in define medium (DN: red histograms), analyzed by multi-parameteric flow cytometry (BD Fortessa). a) & c) graphs represent percentage of expression and b) & d) histograms represent the mean fluorescent intensity (MFI) for each marker expression in U87-MG and LN18 respectively, grey graphs show the isotypes (unlabeled) negative control condition.

B. *GBM cell crossover frequencies*

In order to support these results obtained at the biological level, the two GBM cell lines were characterized by establishing their UHF-DEP crossover frequencies according to their different culture conditions.

The set of crossover frequencies measured for both cell lines (U87-MG and LN18) cultured in the two different conditions (normal culture medium vs. define medium) is represented in the Figure 10 (One should notice that the middle bar here represents the median value of the whole collected data). The considered crossover frequency corresponds to the frequency for which the trapped cell just starts to move away from the center of electrodes quadrupole. Based on statistical analysis of the results observed in the four populations, culturing cells in a define environment seems to have a real impact on the measured

crossover frequencies, according to GBM intracellular characteristics changes. The set of statistics concerning the characterization of U87-MG and LN18 cells crossover frequencies is summarized in Table 1, listing the average, median, standard deviation, standard error, minimum and maximum crossover frequency values for each cell population.

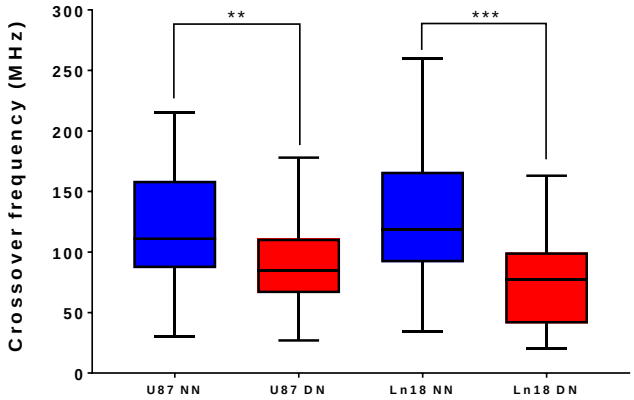


Fig. 10. Graphic box plots representation of U87-MG and LN18 cells crossover frequencies, cultured in two different conditions : normal medium (NN) and define medium (DN). The p value was determined using One-way ANOVA test. *** represents *p* value <.0001, ** represents *p* value <.001.

TABLE I
SUMMARY OF CROSSOVER FREQUENCY MEASUREMENTS (MHZ)

Cell lines	Number of cells	Avg	Median	Dev Std	SEM	Min	Max
U87 NN	104	**120**	111	45.11	4.36	30	215
U87 DN	57	**91**	85	36.34	4.73	27	178
Ln18 NN	138	**128**	119	53.11	4.47	34	260
Ln18 DN	116	**76**	77	34.47	3.17	20	163

As shown a significant number of cells have been characterized showing statistically consolidated data. The large standard deviation, and error standard, observed for U87-MG and LN18 NN cell pool can be explained by the natural cell line heterogeneity: including a large number of different differentiated cells but also some few undifferentiated occurrence in the pool. On the other hand, the DN cell pools may concentrate a much higher number of undifferentiated and low differentiated cells; since DN culture conditions are not favorable for differentiated cell growth.

Considering the measured crossover frequencies, these two GBM cell lines exhibit the same behavior according to the two different culture conditions. Actually, the undifferentiated enriched populations (DN) show much lower crossover frequencies than the cells cultured in normal conditions, although some crossover frequencies overlap exist between these two populations – U87-MG: Average of 120 MHz for NN *vs.* 91 MHz for DN – LN18: Average of 128 MHz for NN *vs.* 76 MHz for DN. This decrease demonstrates a significant difference (illustrated by the run ANOVA statistical analysis tests resulting in a very low *p* value) between these two population profiles obtained by different culture conditions. This finding proves a real difference on the intracellular dielectric characteristics of the undifferentiated cells enriched populations compared to differentiated cells, reflecting the intrinsec biological properties differences.

This difference outlines a great potential for discrimination of cell subpopulations within the whole tumor mass. Therefore such technique is highly promising to achieve discrimination and even isolation of undifferentiated cells allowing potential cell sorting of these undifferentiated subpopulations related to the CSC subpopulation.

V. CONCLUSION

We described here a novel method of cellular subpopulations' discrimination, which completes the classical biological approaches, based on the differential expression levels of a set of markers. These populations, with different cellular differentiation status, are discriminated using real time measurement on microfluidic lab-on-chip (LOC) platform implemented in CMOS technology. Both selected GBM cell lines, showed a strong correlation between the biological markers differences and the measured DEP frequency signatures according to the different culture conditions. Differences on crossover frequencies obtained for each subpopulation, showed a great discrimination potential especially for the development of a novel method to characterize Cancer Stem Cells. Thus, we confirmed the biological differences analyzed by routine methods, using DEP signatures differences, which complete the characterization of stemness properties cells. These results correlate to the biological differences at the functional level. The undifferentiated properties of CSCs are associated to intracellular changes and reflecting their high aggressiveness potential. This technic allows screening of a new cell discrimination parameter, the intracellular differences and physical properties of cells, without any labeling, without affecting cell integrity and viability. Hence, based on the UHF-DEP spectroscopy method, we detected and characterized the undifferentiated cells with unique capabilities to screen biological specificities by investigating the intracellular dielectric properties.

Finally, this method confirms a high potential of emerging lab-on-chip (LOC) platforms in the diagnosis and the treatment of glioblastoma.

ACKNOWLEDGMENT

This project has received funding from the European Union's Horizon 2020 research and innovation program under

grant agreement No 737164.

REFERENCES

[1] M. Cheray, G. Bégaud, E. Deluche, A. Nivet, S. Battu, F. Lalloué, M. Verdier, B. Bessette, "Cancer Stem-Like Cells in Glioblastoma", Editors In: De Vleeschouwer S1, editor. Glioblastoma [Internet]. Brisbane (AU): Codon Publications; 2017 Sep. Chapter 4.

[2] J. Chen, R. M. McKay, L. F. Parada, "Malignant glioma: lessons from genomics, mouse models, and stem cells", Cell. 2012 Mar 30; 149(1): 36-47. doi: 10.1016/j.cell.2012.03.009.

[3] A. Bradshaw, A. Wickremsekera, S. T. Tan, L. Peng, P. F. Davis, T. Itinteang, "Cancer Stem Cell Hierarchy in Glioblastoma Multiforme", Front Surg. 2016 Apr 15;3:21. doi: 10.3389/fsurg.2016.00021. eCollection 2016.

[4] F. Zeppernick, R. Ahmadi, B. Campos, C. Dictus, B. M. Helmke, N. Becker, P. Lichter, A. Unterberg, B. Radlwimmer, C. C. Herold-Mende, "Stem cell marker CD133 affects clinical outcome in glioma patients", Clin Cancer Res. 2008 Jan 1;14(1):123-9. doi: 10.1158/1078-0432.CCR-07-0932.

[5] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, P. Wang, "Cell-based biosensors and their application in biomedicine". Chem. Rev. 2014, 114, 6423–6461.

[6] N. Abd Rahman, F. Ibrahim, B. Yafouz, "Dielectrophoresis for Biomedical Sciences Applications: A review". Sensors 2017, 17, 449.

[7] R. Pethig, Dielectrophoresis: Theory, Methodology and Biological Applications, First published: 25 March 2017, Print ISBN: 9781118671450 |Online ISBN: 9781118671443 |doi:10.1002/9781118671443 Copyright © 2017 John Wiley & Sons, Ltd. All rights reserved.

[8] P. R. C. Gascoyne, J. V. Vykoukal, "Dielectrophoresis-Based Sample Handling in General-Purpose Programmable Diagnostic Instruments", Proc. IEEE Inst. Electr. Electron. Eng. 2004 January 1; 92(1): 22–42. doi:10.1109/JPROC.2003.820535.

[9] E. Salimi, K. Braash, M. Butler, D. J. Thomson and G. E. Bridges, "Dielectric model for Chinese hamster ovary cells obtained by dielectrophoresis cytometry", Biomicrofluidics 10, 014111 (2016); doi: 10.1063/1.4940432.

[10] L.Y. Zhang, C. Bounaix Morand du Puch, C. Dalmay, A. Lacroix, A. Landoulsi, J. Leroy, C. Mélin, F. Lalloué , S. Battu, C. Lautrette , S. Giraud, A. Bessaudou, P. Blondy, M.O.Jauberteau and A. Pothier "Discrimination of Colorectal Cancer Cell Lines using Microwave Biosensors", Sensors & Actuators: A. Physical, Vol 216, 1 Sep 2014, Pages 405-416.

[11] F. Artis, T. Chen, T. Chretiennot,J. J. Fournie, M. Poupot, D. Dubuc, K. Grenier, , "Microwaving Biological Cells: Intracellular Analysis with Microwave Dielectric Spectroscopy," IEEE Microwave Magazine, vol. 16, no. 4, pp. 87-96, May 2015.

[12] X. Ma, X. Du, H. Li, X. Cheng and J. C. M. Hwang, "Ultra-Wideband Impedance Spectroscopy of a Live Biological Cell," in IEEE Transactions on Microwave Theory and Techniques. doi: 10.1109/TMTT.2018.2851251 .

[13] H. A. Pohl, "The Motion and Precipitation of Suspensoids in Divergent Electric Fields". J. Appl. Phys. **1951**, 22, 869–871.

[14] F. Hjeij, C. Dalmay, A. Bessaudou, Pierre Blondy, Arnaud Pothier, B. Bessette, G. Begaud, M.O. Jauberteau, F. Lalloue, C. Baristiran Kaynak, Mehmet Kaynak, C. Palego, "UHF Dielectrophoretic Handling of Individual Biological Cells Using BiCMOS Microfluidic RF-Sensors, 46th European Microwave Conference (EuMC),London, October 2016.

[15] G. S. Fiorini, D. T. Chiu, "Disposable microfluidic devices: Fabrication, function, and application. BioTechniques", **2005**, 38, 429–446.

[16] L. M. Broche, F. H. Labeed and M. P. Hughes, "Extraction of dielectric properties of multiple populations from dielectrophoretic collection spectrum data", Phys. Med. Biol. 50, 2005, 2267-2274.

[17] X. Du, X. Ma, H. Li, Y. Ning, X. Cheng, and J. C. M. Hwang, "Validation of Clausius-Mossotti Function in Single-Cell Dielectrophoresis", 2018 IEEE/MTT-S International Microwave Biomedical Conference.

[18] K. Asami, Y. Takahashi and S. Takashima, "Dielectric properties of mouse lymphocytes and erythrocytes", Biochimica et Biophysica Acta, 1010 (1989) 49-55.

[19] S. Afshar, A. Fazelkhah, E. Salimi, M. Butler, D.Thomson, G. Bridges, "Change in the Dielectric Response of Single Cells Induced by Nutrient Deprivation Over a Wide Frequency Range", 2017 IEEE MTT-S International Microwave Symposium (IMS).

[20] J. Gimsa, P. Marszalek, U. Loewe and T. Y. Tsong, "Dielectrophorsis and electrorotation of neurospora slime and murine myeloma cells", Biophys., J.60, 749-760, 1991.

[21] C. Chung, R. Pethig, S. Smith, M. Waterfall, "Intracellular potassium under osmotic stress determines the dielectrophoresis cross-over frequency of murine myeloma cells in the MHz range", Electrophoresis 2018, 39, 989-997.