

# Ultra High Frequency Dielectrophoresis Manipulation to Monitor the Kinetics of Glioblastoma Cells Stemness Phenotype Acquirement

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**Abstract**—This paper demonstrates the great potential of Ultra-High Frequency dielectrophoresis (UHF-DEP) to monitor the kinetics of glioblastoma (GBM) cells stemness phenotype transformation. Above 20 MHz, the method is able to probe the intracellular content and to be sensitive to its conductivity and permittivity values. U87-MG GBM cell line was cultured in different conditions in order to induce a differentiation gradient phenotype among the cell population. Using the presented characterization technic, it is shown that undifferentiated cells can be discriminated.

**Keywords**—Ultra High Frequency Dielectrophoresis, Cell electro-manipulation, glioblastoma cells, cancer stem cells

## I. INTRODUCTION

Glioblastoma (GBM) is a grade IV highly aggressive brain tumor. It represents one of the most difficult disease to treat due to some hidden tumor initiating cells with an undifferentiated phenotype, called cancer stem cells (CSCs) [1]. This small cell subpopulation is suspected to be responsible of tumor relapse after complete remission and of therapeutic resistance mediation by displaying an undifferentiated phenotype [2]. GBM presents a highly cellular and morphological heterogeneity inside the tumor and cancer cells might show different grades of differentiation grades [1]. This tumor heterogeneity results in a therapeutic challenge to improve the care of the disease by the detection of CSCs. To do so, biologists currently use immunofluorescent labels coupled with flow cytometry. However, one of the main issue is that common biomarkers can be also expressed by differentiated tumor cells or cells following a differentiation process [3].

Hence, we take advantage of a label-free method called dielectrophoresis (DEP) to discriminate CSCs from differentiated cells. Indeed, at ultra-high frequency (UHF)

range, above 20MHz, we are able to characterize the intracellular content and measure physical properties relying on its intrinsic dielectric specificities. In this paper, we present a microfluidic lab-on-chip based on UHF-DEP able to follow the kinetics of the undifferentiated phenotype acquirement of glioblastoma cells.

## II. PRINCIPLE OF CELL'S ELECTROMANIPULATION

### A. Basic DEP theory

Biological cells can be individually electro-manipulated under an applied field, as they become polarizable. Indeed, DEP principle relies on the redistribution of the charges at the interface between the cell and the suspension medium. The imbalance introduced by the non-uniformity of the electric field, generate DEP displacement force on the cell which can be written as follow [4]:

$$F_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}[f_{cm}(\omega)] \nabla E^2 \quad (1)$$

Where  $r$  is the cell radius,  $\varepsilon_m$  the permittivity of the suspension medium,  $f_{cm}$  the Clausius-Mossotti (CM) factor and  $E$  the applied electric field. The CM factor describes the polarization state of the biological cell in a suspension medium which is linked to their dielectric properties (permittivity and conductivity). Its real part determines the direction of the cell's motion (Fig. 1A). If the real part is positive, the DEP force is attractive and the cell presents what we call a positive DEP (pDEP) behavior. It means that the cell will be pulled toward strong field areas, that is to say toward one edge of the electrode (in red in Fig. 1B). On the contrary, if the real part of the CM factor is negative, the DEP force is repulsive and the cell will be in a negative DEP (nDEP) behavior. Actually, it will be repelled to the weak field areas, i.e. to the center of the quadrupole (in blue). The behavior alternation between pDEP

and nDEP occurs at the crossover frequency  $f_{x02}$ , where the cell is not submitted to the DEP force anymore.

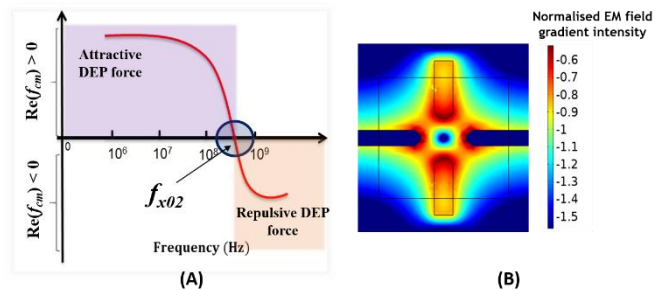


Fig. 1. Principle of UHF crossover frequency measurement. (A) Graph of the real part of the CM factor. (B) Numerical simulation of the biased quadrupole in a non-uniform electric field.

The crossover frequency  $f_{x02}$  appears at high frequency regime (above 50 MHz). At this range, the electromagnetic wave can penetrate inside the biological cell and interact with the intracellular content. We have previously identified the UHF range as a relevant electromagnetic biomarker to discriminate undifferentiated cells from differentiated one in the case of glioblastoma [5].

### B. Crossover frequency measurement method

To determine the UHF-DEP signature, we use a specific quadrupole sensor implemented on a microfluidic chip BiCMOS (Fig. 2), allowing the individual cell electro-manipulation. The cell suspension is injected into the Lab-On-Chip and can circulate inside a microfluidic channel. By applying pressure thanks to external flow controllers (Elveflow OB1), cells' speed can be adjusted adequately above the sensor. The crossover frequency is measured with a device made with four electrodes, biased with a high frequency continuous wave (CW) signal thanks to 50Ω coplanar waveguides, connected to RF probes (MPI TITAN T26P-GSG-150). Our microfluidic chip allows an efficient single-cell trapping thanks to the electrical cage created by the quadrupole sensor (Fig. 1B).

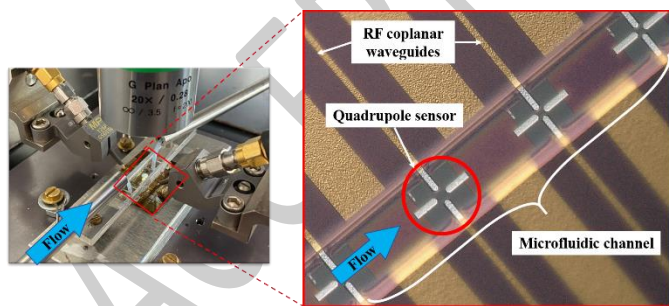


Fig. 2. Biased microfluidic DEP chip connected to cell suspension and zoom on the quadrupole microelectrodes sensor.

The method for UHF-DEP characterization is deeply described in [5], [6]. When a cell arrives around the center of sensor, the cell suspension flow is stopped and stabilized. Then, the electric field is switched on at a frequency of 500MHz (far above the crossover frequency  $f_{x02}$ ) to ensure the cell reaction in nDEP behavior. Consequently, the cell is repulsed and trapped at the center of the quadrupole. Then, the DEP signal

frequency progressively decreases until observing the cell attraction toward one edge of the electrode. From the optical monitoring of the cell motion, we can measure the second crossover frequency.

## III. EXPERIMENTAL RESULTS

### A. Cell preparation

The cell line used in this paper has been purchase from American Type Culture Collection (ATCC). U87-MG is a glioblastoma cell line derived from an adult brain tumor of grade IV. Cells were cultured in different conditions in order to enrich in CSCs:

- Normal Medium (NM): induces cell differentiation in DMEM supplemented by 10% FBS, 2mM glutamine and 1% penicillin/streptomycin.
- Define Medium (DM): induces stringent conditions for CSCs enrichment in selective DMEM/F12 supplemented by 0.6% sucrose, 1% sodium bicarbonate, 1% MEM non-essential amino acids, 5mM HEPES, 9.6μg/mL putrescine, 10μg/mL ITSS, 0.063μg/mL progesterone, 60μg/mL N-acetyl-L-cysteine, 2μg/mL heparin, 0.1mg/mL penicillin/streptomycin, 50X B-27 supplement without vitamin A, 20ng/mL EGF, 20ng/mL FGF2.

Cell were cultured in appropriate flasks and maintained at 37°C in a humidified 5% CO<sub>2</sub> – 95% air incubator. So, U87-MG cell line was characterized under three conditions: (i) 6 days in normal medium (NM); (ii) 5 days in define medium (DM); (iii) maintained during 21 days in define medium (DM+). Indeed, we expect that in DM condition, cells presenting a stemness-like phenotype will be selected.

Before DEP characterization, U87-MG cells were suspended in an osmotic medium adapted for DEP experiments made from deionized water (ion free) supplemented by sucrose (conductivity: 26 mS/m and pH: 7.6).

### B. Biological analysis of GBM cell line phenotype

In order to attest the enrichment of undifferentiated cells in DM condition, a comparative transcriptomic analysis of the stemness phenotype was performed to assess the mRNA expression levels related to CSCs biomarkers (*CD133*, *Nanog*, *Sox2* and *Oct4*).

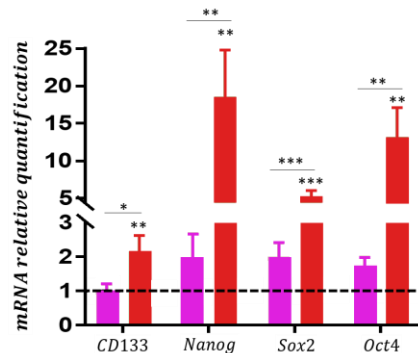


Fig. 3. Comparative analysis of gene expression of four CSCs biomarkers: *CD133*, *Nanog*, *Sox2* and *Oct4* among U87-MG cell line, cultured in NM condition (dotted line), or in DM condition (in purple), or in DM+ condition (in red).

red), measured by Real Time PCR. \*\*\* represents p-value < 0.001, \*\* p-value < 0.01 and \* p-value < 0.05.

In Fig. 3, mRNA expression levels of U87-MG cultured in DM (purple bars) and DM+ (red bars) conditions were normalized respectively to NM condition (dotted line). The analyzed CSCs biomarkers show an overexpression only in DM+ condition, confirming the presence of CSC in define medium. We can see that in DM condition, the transcripts are less expressed than in DM+ condition, this mean that the cell population is a mix between differentiated and undifferentiated cells.

### C. Crossover frequency results

The U87-MG cell line has been characterized using the microfluidic lab-on-chip described in Fig. 2. The results of different culture conditions are reported in Fig. 4. One can notice that each point on the box plot relates to the signature measurement of one cell. The color box gathers the crossover frequencies of 50% of the investigated cells. When cells are maintained a long time in stringent conditions (DM+ condition), they tend to form gliomaspheres to survive which might cause necrosis [2]. Hence, cell viability is low and so less cells can be characterized.

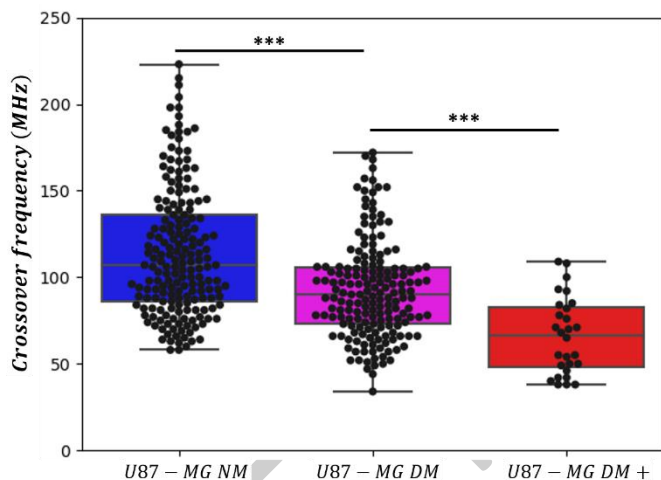


Fig. 4. Graphic box plot representation of U87-MG cells crossover frequencies cultured in three different conditions: (i) 6 days in normal medium (NM); (ii) 5 days in define medium (DM); (iii) 21 days in define medium (DM+). \*\*\* represents p-value < 0.001.

One can notice that the measured crossover frequencies of the different populations are dispersed on a more or less frequency range for NM and DM conditions. This observation highlights the natural biological heterogeneity present among the GBM U87-MG cell line [1]. In normal medium, the median value of  $f_{x02}$  is 108 MHz, after 5 days in define medium, the DEP signature decreases to 88 MHz. When the cells are maintained in DM+ condition, the median value drops to 67 MHz. Table I reports the ratio of measured  $f_{x02}$  values observed for several frequency ranges according to the culture condition.

TABLE I. SUMMARY OF THE CROSSOVER FREQUENCY REPARTITION ACCORDING TO THE DIFFERENT CULTURE CONDITIONS

Frequency range/Culture condition	NM	DM	DM+
$f < 50\text{MHz}$	0%	3%	36%
$50\text{MHz} < f < 100\text{ MHz}$	44%	62%	57%
$100\text{MHz} < f < 150\text{ MHz}$	38%	30%	7%
$150\text{MHz} < f < 200\text{ MHz}$	16%	5%	0%
$200\text{MHz} < f$	2%	0%	0%

According to the data of Table I, in NM condition, 56% of the characterized cells display a crossover frequency higher than 100 MHz whereas in DM+ condition, only 7% of the cell population shows this behavior. Hence, this crossover frequency distribution points out that despite an overlap between each condition, cells that are able to survive under stringent conditions exhibit a lower crossover frequency. Moreover, the DM cell pool concentrates 92% of its population between 50 MHz and 150 MHz at a medium frequency range. In correlation with the previous biological analysis, it seems that GBM cells owning a transition state that follows an undifferentiating process can be found in this frequency range. Thus, the proposed microfluidic system is able to monitor the kinetics of U87-MG stemness phenotype acquirement in a CSC enrichment medium.

### IV. CONCLUSION

At ultra-high frequency range, the electromagnetic wave is able to interact with the biological internal properties of cells and then, we can exploit their UHF-DEP signature to have an information about their stemness status. Indeed, cells presenting a stemness-like phenotype has a high probability to be identified by a lower UHF-DEP signature. Our microsystem offers possibilities to track and follow the kinetics of the undifferentiated process. This technic, in the near future, could be a relevant tool to help biologists to optimize GBM care with an efficient therapeutic strategy.

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