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UHF-Dielectrophoresis Signatures as a Relevant Discriminant Electromagnetic Biomarker of Colorectal Cancer Stem Cells

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Abstract—Nowadays, cancer stem cell (CSC) characterization with an efficient, fast and low-cost method still remains an issue. Hence, we have developed a microfluidic lab-on-chip RF sensor based on dielectrophoresis (DEP) to characterize individual biological cells by measuring their two crossover frequencies: f_{x01} (low frequency regime, below 1 MHz) and f_{x02} (Ultra High Frequency regime, above 50MHz). These electromagnetic signatures have been determined and compared between cancer stem cells and tumor differentiated cells from colorectal cancer cell line. We show that representative crossover frequency change appears in the UHF regime making such signatures a relevant biomarker to identify cancer stem cells among a tumor population.

Keywords— Radiofrequency biosensors, UHF dielectrophoresis, lab-on-chip, biological cell analysis, cancer diagnosis.

I. INTRODUCTION

Treatment resistance is a major issue in many cancers and especially in the management of colorectal cancer (CRC). One of the hypotheses to explain this treatment failure is based on the emergence of cancer stem cells (CSCs) able to resist to radio- and chemo-therapeutic treatments. The undifferentiated phenotype of CSCs [1] could explain the maintenance of the minimal residual disease able to regenerate the tumor mass, which would favor recurrences. Nowadays, the detection of CSCs from patient tumor biopsy using biomarkers is crucial to establish the prognostic value on the therapeutic response in order to adapt the disease's management [2]. A biomarker can be any measurable biological indicator able to recognize the target population. It can be of different types: cellular, molecular (protein, antigen, mRNA...) by using labels or based on physiological and morphological features. Physicians from pathology departments are used to apply histological staining or specific molecular characterization to identify pathological cells within surgical samples. However, immunofluorescence detection of CSCs remains difficult and can only be carried out using some generic labels that can also be expressed by other cancer cells. The lack of CSC specific biomarkers is a main issue, especially since the number of CSCs within the tumor is limited which makes their detection even more complex. [3]. As a result, to support the evidence of CSC occurrence, biologists analyze a wide panel of biomarkers, involving expensive and time-consuming staining steps. Moreover, this

approach must be complemented by functional tests such as clonogenicity, to prove CSC's capability as single cell to generate a tumor embryo [4]. However, such experiments require up to 20 to 40 days of process and are so not sustainable to be implemented in clinical routine.

In this paper, we propose an alternative and complementary approach based on identification of intrinsic dielectric specificity of CSCs versus tumor differentiated ones. We have developed a microfluidic lab-on-chip sensor (Fig. 1) able though a label-free, fast and low-invasive characterization method, called dielectrophoresis (DEP) performed in the UHF frequency range, to assist biologists. Indeed, we can provide diagnosis results within few hours, compare to approximately 20 days currently using functional tests as clonogenic assay. In this context, we aim to investigate and compare the relevance of two electromagnetic biomarkers at low frequency (below 1MHz) and high frequency (above 50MHz) with our RF-lab-on-chip for the identification of treatment-resistant CSCs within the tumor.

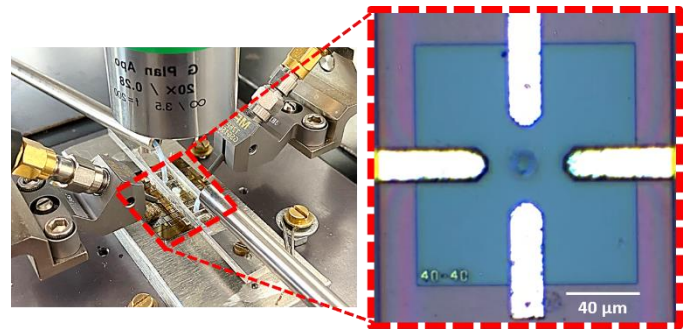


Fig. 1. Quadrupole RF sensor implemented on a microfluidic chip biased with a UHF CW DEP signal for single cell characterization.

II. CELL CHARACTERIZATION BY DIELECTROPHORESIS

The manipulation of individual biological cells using dielectrophoresis is practiced since two decades at low frequency regime and is well mastered [5]. The main principle involves the induction of a dielectrophoretic force F_{DEP} that can induce the cell motion once submitted to a non-uniform electric field.

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

where $\epsilon_{p,m}^*$ are the complex permittivity of the particle and of the medium respectively, ϵ_m is the real part of ϵ_m^* , r the radius of the cell, ω the angular frequency of the signal, E the applied electric field and $\text{Re}[f_{cm}(\omega)]$ the real part of the Clausius-Mossotti (CM) factor described as follow [6]:

$$f_{cm}(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

The CM factor is frequency dependent. It relies on the polarization state of the cell in its suspension medium and thus, determines the orientation and the magnitude of the DEP force according to the frequency of the applied signal. As illustrated in Fig. 2a at low frequency, the DEP force is repulsive, due to the negative DEP (nDEP) behavior of cell, inducing its motion toward weak electric field areas. As the frequency increases, $\text{Re}[f_{cm}]$ becomes positive, inducing a positive DEP (pDEP) behavior which results in an attractive force, moving the cell toward strong field areas. Two crossover frequencies: f_{x01} at low frequency and f_{x02} at high frequency, mark the limit of the switching of motion behavior.

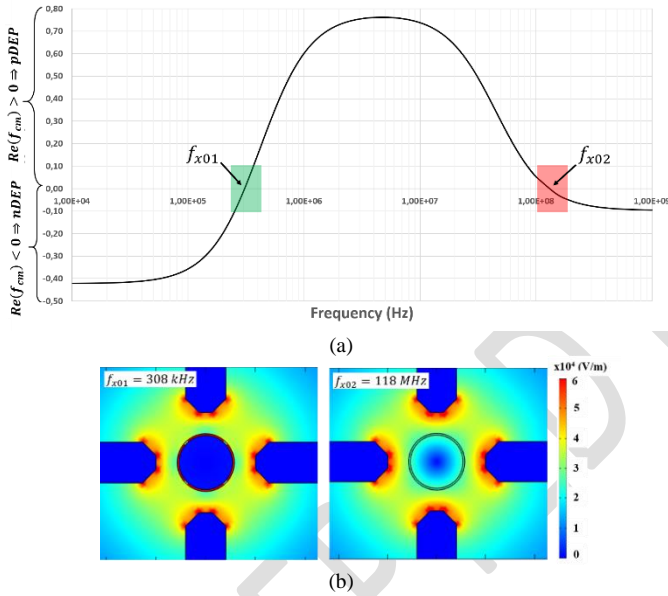


Fig. 2. Numerical simulation of a cell dielectric behavior as function of the frequency considering the Table 1 parameters. (a) Plot of the real part of the Clausius-Mossotti factor ; (b) COMSOL Multiphysics simulation (at the two crossover frequencies) of the electric field disturbances due to the cell's presence. The color scale corresponds to the electric field intensity (V/m).

Actually these crossover frequencies can be expressed as [6]:

$$f_{x01} = \sigma_m \frac{th_{cm}}{\sqrt{2\pi r} \epsilon_{cm}} \quad f_{x02} = \frac{\sigma_{int}}{2\pi} \sqrt{\frac{1}{2\epsilon_m^2 - \epsilon_{int}\epsilon_m - \epsilon_{int}^2}} \quad (4)$$

where σ_m is the conductivity of the suspension medium, th_{cm} and ϵ_{cm} respectively the thickness and the permittivity of the cell membrane. The crossover frequency f_{x01} both relies on the dielectric properties of the plasma membrane and on the cell's size, morphology and shape. Whereas, the second crossover frequency f_{x02} depends essentially on the intracellular content and the overall dielectric features of the cytoplasm.

Fig 1b shows the influence of a cell on the electric field set alternately at the two crossover frequencies, both computed

with (4). The simulation was performed with COMSOL Multiphysics software considering the parameters of Table 1. One can notice that at f_{x01} , the electric field is mostly concentrated inside the membrane which acts as an insulator. Actually, it has been shown that lower frequency regime may translate changes of membrane properties occurring between different cell types according to their size [8] or their viability [9]. At f_{x02} , the electromagnetic wave can interact with the intracellular content. Such content should differ between cells with different level of maturity especially from stem cells to fully differentiated ones. It is so expected that such cells may present different dielectric properties and thus two distinct second crossover frequencies [10].

Table 1. Values of the different dielectric and cellular parameters used in COMSOL Multiphysics simulations. (NB: membrane thickness was increased to avoid mesh issues, hence membrane properties were modified proportionally to respect cell dielectric behavior).

Parameters	Value
Particle radius	11.5 μm
Membrane thickness	700 nm
Membrane relative permittivity	100 $\epsilon_0 \text{ F.m}^{-1}$
Membrane conductivity	1.43 e-4 S/m ²
Intern relative permittivity	50 $\epsilon_0 \text{ F.m}^{-1}$
Intern conductivity	0.5 S/m
Medium relative permittivity	78 $\epsilon_0 \text{ F.m}^{-1}$
Medium conductivity	0.02 S/m

In this context, we already demonstrate the relevance of using UHF regime to detect the stemness phenotype in the case of glioblastoma [11]. This paper aims to strengthen the use of UHF-DEP as a relevant approach for discriminating the undifferentiated from the differentiated phenotypes. The reproducibility of the method will be proved by characterizing a different cell line which originates from colorectal cancer. Therefore, the two crossover frequencies f_{x01} and f_{x02} of tumor differentiated cells and cancer stem cells will be measured and compared.

III. EXPERIMENTAL PROTOCOLS

A. Crossover frequencies f_{x01} and f_{x02} measurement

The cell characterization is performed with the measurement of the two crossover frequencies for both CSCs and tumor differentiated cells from a CRC cell line. The characterization method is detailed in [11]. A quadrupole sensor was implemented on a silicon microchip as show in Fig 1. Cells are flowing to the characterization area thanks to a dedicated microfluidic channel. The electrodes create an electrical cage in which a single cell can be captured. Once the electrode biased, the signal frequency can be tuned to observe the cell's motion in order to measure both f_{x01} and f_{x02} crossover frequency values.

B. Cell preparation

The presented experiments were performed with SW620 cells: a colon cancer cell line isolated from a lymph node which has metastatic origin. This cell line was purchased from American Type Culture Collection (ATCC) and cultured in

such way to induce two different phenotypes: common (differentiated) tumor cells using a Normal culture Medium (NM) which promotes cell differentiation; and CSC-like (undifferentiated) cells thanks to a stringent Define culture Medium (DM). Indeed, culturing cells in these conditions, improper to differentiated cell survival, favors the selection of immature cells and thus allow enriching the cell pool with CSCs. For both conditions, cells were cultured during 6 days before being characterized.

IV. EXPERIMENTAL RESULTS

A. Biological analysis of cell line phenotype

To evaluate the enrichment of undifferentiated cells for subpopulation cultured in Define Medium, the transcriptomic mRNA expression levels related to conventionally considered CSCs related intracellular immuno-markers (*CD133*, *Nanog*, *Sox2*, *Oct4*, *Bmi1* and *Lgr5*) have been compared between NM and DM cultured cells. In Fig. 3, mRNA relative quantification of SW620 cultured in DM were normalized respectively to SW620 cultured in NM (dotted line). Analyzed CSCs transcripts show a clear overexpression of only three CSC markers (*CD133*, *Nanog* and *Oct4*) in DM conditions while unexpected *Sox2* and *Lgr5* results are more difficult to read, highlighting the previously discussed limitation of using generic biomarkers.

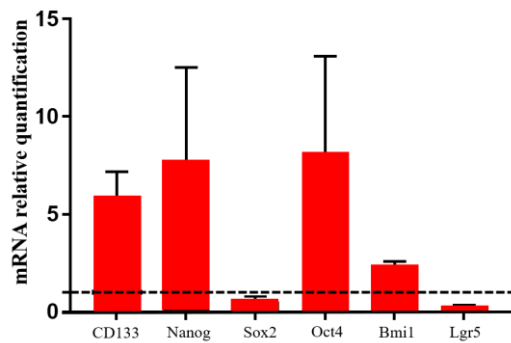


Fig. 3. Comparative analysis of gene expression of five markers of undifferentiation measured by Real Time PCR: *CD133*, *Nanog*, *Sox2*, *Oct4*, *Bmi1* and *Lgr5* among SW620 cell line, cultured in Normal Medium (dotted line as normalization reference), or in Define Medium (DM).

B. Correlation with the crossover frequencies

These differentiated and immature SW620 sub-populations has been also characterized with the proposed dielectrophoresis method, by measuring their two crossover frequencies f_{x01} and f_{x02} in low and high frequency regime. The results for the two culture conditions are summarized in the violin shape plot in Fig 4. This graph illustrates how the different measured crossover frequencies of characterized cells are distributed. The width of the figure is proportional with the number of characterized cells owning the same crossover frequency value. One can notice that for the four datasets the measured crossover frequencies are spread on few hundreds of kHz or MHz respectively for f_{x01} and f_{x02} , which is expected and reflects on the intrinsic biological heterogeneity existing between the cells

of these populations. Nonetheless, 50% of cell population is concentrated in a more restricted frequency range (black rectangle) around the median crossover frequency values of the dataset (white dot). Such range can be considered as the representative DEP signature of the population.

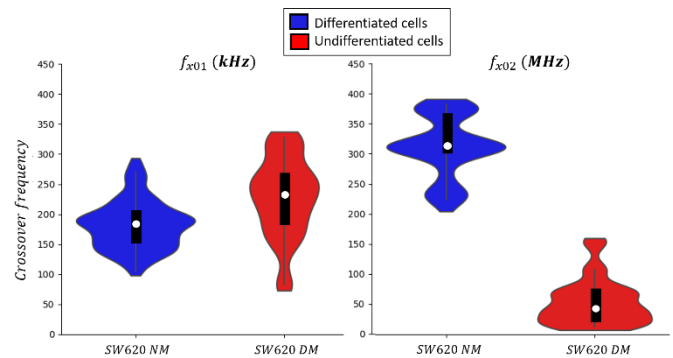


Fig. 4. Graphic violin plot representation of SW620 cells crossover frequencies f_{x01} (left graph) and f_{x02} (right graph), cultured in two different conditions: Normal Medium (NM) and Define Medium (DM).

Actually, the measured f_{x01} median values are 184kHz and 228kHz respectively for NM and DM conditions. 50% of measured crossover frequencies ranges between 149kHz and 204kHz for NM and between 176kHz and 280kHz for DM, resulting in a large overlap between both signatures. Consequently, at low frequency, the measured dielectric property difference between the two cell populations is not enough significant to be exploited as a discriminant biomarker. However, at high frequency, the DM cell pool shows a major decrease of their f_{x02} with a median value of 55MHz related to the 313MHz one for NM cells. In addition, 50% of measured crossover frequencies ranges between 305MHz and 365MHz for NM versus between 19MHz and 75MHz for DN cells, displaying very distinct signatures. These results illustrate that using f_{x02} measurements is very relevant to identify stem cell like cells and can be considered as a new electromagnetic biomarker of CSCs. This demonstrates that characterizing biological cells in UHF regime is a good strategy, especially to allow label-free detection of undifferentiated cells by identifying their intracellular dielectric property specificities.

V. CONCLUSION

This paper illustrates how measuring UHF-DEP signature can be a relevant approach to establish new electromagnetic biomarker for the detection of CSCs among a heterogeneous tumor population. Such innovative characterization method might also help to identify treatments that specifically target CSCs in order to eradicate this resistant cell population and could be used to analyze the efficiency of such drugs specifically on patient tumor cells in order to choose the best personalized therapeutic strategy.

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