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

S. Saada[1], R. Manczak[2], B. Bessette[1] , C. Dalmay[2], G. Begaud[1], S. Battu[1], P. Blondy[2], M.O. Jauberteau[1], M. Inac[3], C. Baristiran Kaynak[3], M. Kaynak[3], C. Palego[4], A. Pothier[2] and F. Lalloue[1]

1- EA 3842-CAPTuR, University of Limoges, 87025 Limoges, France; 2- XLIM-UMR 7252, University of imoges/CNRS, 87060 Limoges, France; 3- IHP Microelectronics, 15236 Frankfurt (Oder), Germany, 4- Bangor University, Bangor, LL57 1UT, Unite Kingdom

Horizon 2020

Introduction : We are introducing here firsts results of glioblastoma cell lines characterization; measuring their crossover frequencies by dielectrophoresis (DEP) technics in the UHF frequency range (above 50 MHz). LN18 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 define 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.

Background : Dielectrophoresis (DEP) induces motion of particles submitted to a non-uniform electric field when the particles and surrounding medium have different polarizabilities. In the present case, regarding the used sensor electrode geometry by changing the frequency of the applied electric field, the polarized particles would array in various motions; which relies on the difference of polarizability between the particle and its surrounding medium.

Neutral particle being exposed to a non-uniform electric field, showing the direction for **Pos**- and **Neg-** DEP

(N. M. Jesu´s-Pe´rez and B. H. Lapizco-Encinas Electrophoresis 2011)

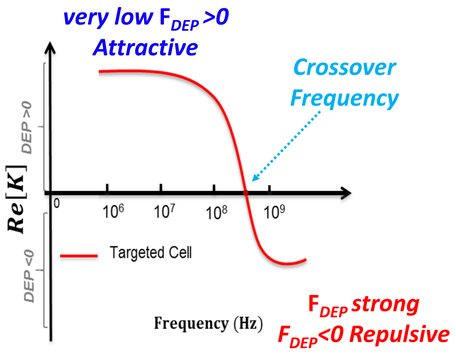
Quadrupole electrode sensor

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

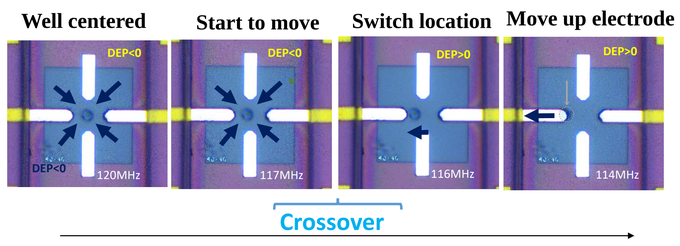
➔ F_{DEP} **high** in **strong field** areas

➔ F_{DEP} **low** in **weak field** areas

DEP spectrum (Re[K(ω)] vs. frequency) of a polarizable particle. When σp < σm and εp >εm; (B) when σp > σm and εp < εm.- ε and σ represent conductivity and permittivity of particles and medium, respectively.

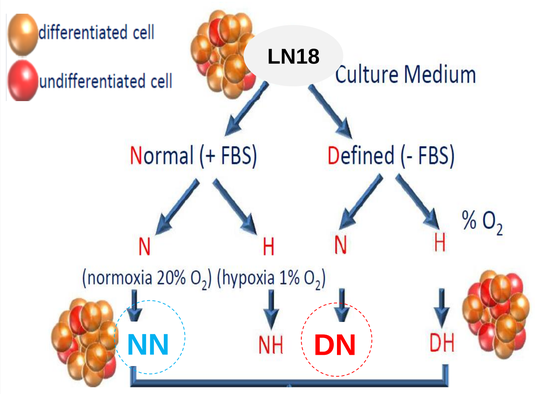


Measurement strategy :
Cell response crossover frequency

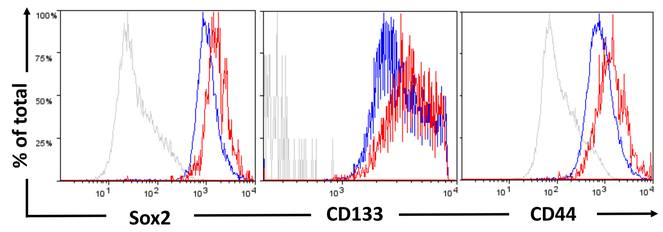


Frequencies increases above several tens of MHz, the plasma membrane lets the electric field penetrate the cell and interact directly with the cell interior. Thus, cell content differences and properties, which reflecting, different cellular and biological properties and specificities mechanisms.

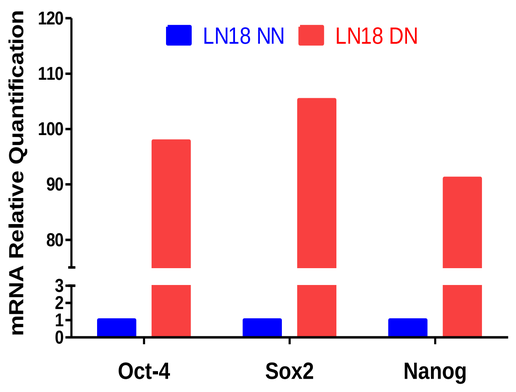
Cancer cell culture conditions

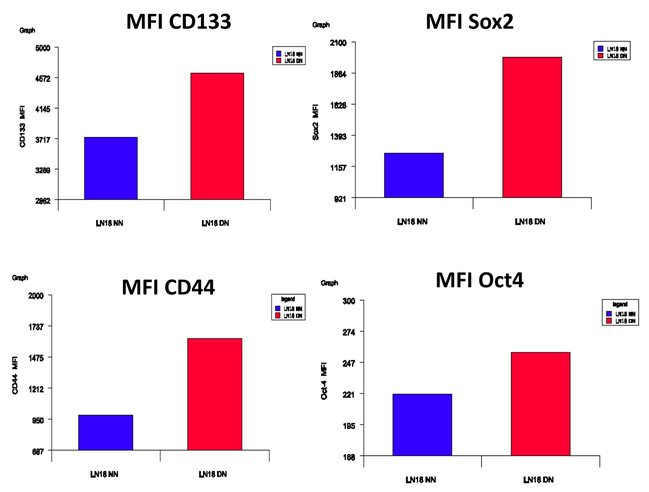


Flow Cytometry Analysis

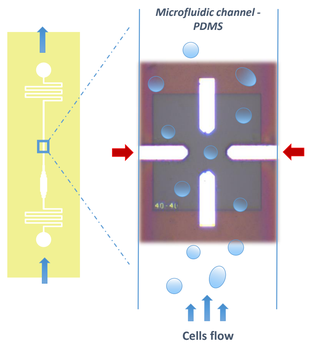


Real Time PCR

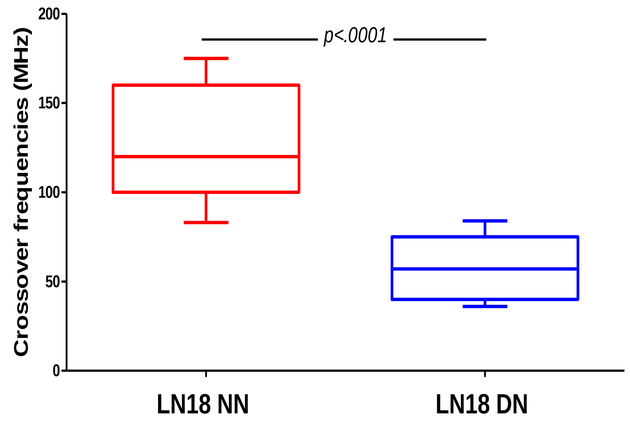




Overexpression of CSCs markers (CD133, CD44, Oct-4, Sox2 and Nanog) in LN18 cultured in define medium (DN), compared to Normal medium (NN), after 6 days culture in normoxia. The CSCs markers were analyzed in LN18 cell line, cultured in each condition. These are measured at protein level by Flow cytometry and at transcriptional level by real time PCR.



Measure



Significant differences of crossover frequencies in LN18 cells, cultured in defined medium (DM condition), which allows CSCs enrichment and in differentiated condition normal medium (NM condition). The p value was determined by t test.

Conclusion : We demonstrated 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 charracteristics and differences, based on 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. These results are well 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 developpement of new glioblastoma therapeutics.

This project has received funding from the European Union's Horizon 2020 Framework Programme FET OPEN Research and innovation under grant agreement N° 737164

This work was presented at the 11th Internationa Conference on ''***Cancer Stem Cells and Oncology Research***'', Jun 11-13 in Dublin, Ireland