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Ultra-High Frequencies continuous biological cell sorting based on repulsive and low dielectrophoresis forces

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***Abstract*— This paper demonstrates the superior capabilities of Ultra-High Frequency dielectrophoresis (UHF-DEP) to sort populations of biological cells based on their intracellular dielectric characteristics. The proposed concept combines both hydrofluidic and repulsive dielectrophoresis forces into a microfluidic lab-on-chip to create a UHF-DEP cytometer. The main objective is to sort different types of cells using only negative dielectrophoresis principle. The idea is to select proper frequency for the applied electric field in order to produce different intensity of repulsive DEP forces related to the cell type. This sorting principle, without positive DEP, limits strong interaction of cells with the electric field, which could induce their permanent trapping during cytometer operation and reduces the efficiency of the cell sorting. Results presented in this paper demonstrate the capability of an effective sorting for mesenchymal cells.**

***Keywords*—microfluidics, high frequencies, dielectrophoresis, biological cells.**

I. INTRODUCTION

In the biomedical field, the sorting of cells is prime of importance in a large range of applications including the development of diagnostic and therapeutic tools but also in cellular biology. In recent years, with the progress of microfluidics and lab-on-chip technologies, researchers have focused their attention on the development of novel label free cell sorting technics. The main motivation is to avoid any type of labels, like biochemical molecules, metallic or magnetic beads that can modify the biological cell to analyze but also to make experimentations less expensive, complex and time consuming.

Label free cell sorting technics are non-invasive methods based on cells physical characteristics, like their size, density, optical or dielectric properties [1]. In this context, dielectrophoresis (DEP) technics appears very attractive.

Indeed, the DEP principle is based on the interaction between a dielectric particle, here a biological cell, and a non-uniform electric field, which generate displacement forces on cells. This technic has been largely explored particularly in the 10kHz to 10MHz frequency range and its efficiency to sort cells especially based on their size and membrane properties has been demonstrated [2].

This paper is focused on Ultra-high Frequencies dielectrophoresis (UHF-DEP) working in the hundreds of MHz frequency range. Recently, some research works using UHF-DEP have been led especially due to the fact that high frequencies allows bypassing the cell membrane. Consequently, generated DEP forces are strongly linked to the intracellular properties [3-5] more than the cell membrane properties.

The objective of this paper is to develop a new generation of UHF-DEP cell cytometer as the prototype shown in Fig.1. The proposed sorting principle is based on the use of repulsive UHF-DEP forces as it will be discussed in Section II. Cytometer design validation is achieved in Section III, where first experimental results on mesenchymal cells are presented.

II. PRINCIPLE AND CYTOMETER DESIGN

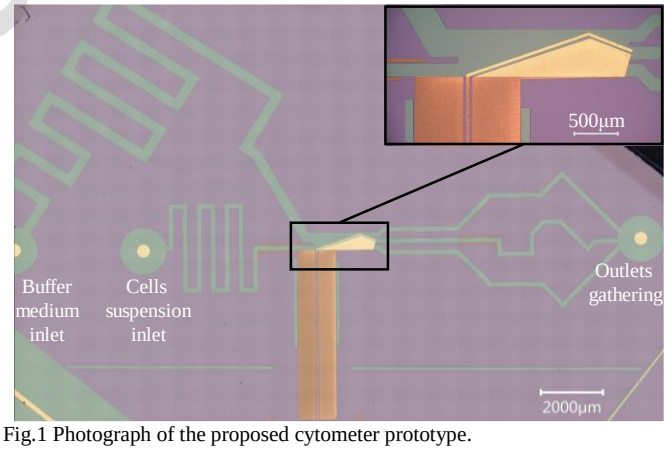

Fig.1 Photograph of the proposed cytometer prototype.

A. Cell interaction with electric field

The proposed cell sorting concept takes advantage of the electromagnetic field effects on individual biological cells. Indeed, when cells are suspended in a low or moderate conductive aqueous medium and submitted to a non-uniform electric field (E field), they become polarized particles and are subject to a dipole moment. Therefore, depending on their own dielectric properties related to their suspension medium ones, cells undergo more or less strong dielectrophoresis forces that can make them moving [6]. The magnitude of these forces can be computed using (1):

$$F_{DEP} = 2\pi\varepsilon_m f_{CM} r^3 \nabla |E_{rms}|^2 \quad (1)$$

Where ε_m is the dielectric permittivity of the cell suspension medium in which cells are suspended, $\nabla|E_{rms}|$ is the gradient of the electric field, r the radius of the cell and f_{CM} the Clausius-Mossotti factor (CMF) which is given by (2):

$$f_{CM} = \frac{\varepsilon^*_p - \varepsilon^*_m}{\varepsilon^*_p + 2\,\varepsilon^*_m} \quad (2)$$

Where ε^*_m and ε^*_p are respectively the complex permittivity of the suspension medium and the cells. Considering cells as a uniform dielectric spheres surrounded by an insulating membrane, ε^*_p can be expressed by (3):

$$\varepsilon^*_p = \varepsilon^*_{mem}\left[\frac{\left(\frac{r}{r-d}\right)^3 + 2\frac{\varepsilon^*_{int} - \varepsilon^*_{mem}}{\varepsilon^*_{int} + 2\,\varepsilon^*_{mem}}}{\left(\frac{r}{r-d}\right)^3 - \frac{\varepsilon^*_{int} - \varepsilon^*_{mem}}{\varepsilon^*_{int} + 2\,\varepsilon^*_{mem}}}\right] \quad (3)$$

Where d is the thickness of the plasma membrane, ε^*_{int} and ε^*_{mem} are respectively the complex permittivity of the intracellular content, and the membrane. The complex permittivity is given by (4):

$$\varepsilon^*_x = \varepsilon_x - \frac{j}{\omega}\sigma_x \quad (4)$$

Where ε_x is the absolute permittivity, σ_x is the conductivity and ω the angular frequency.

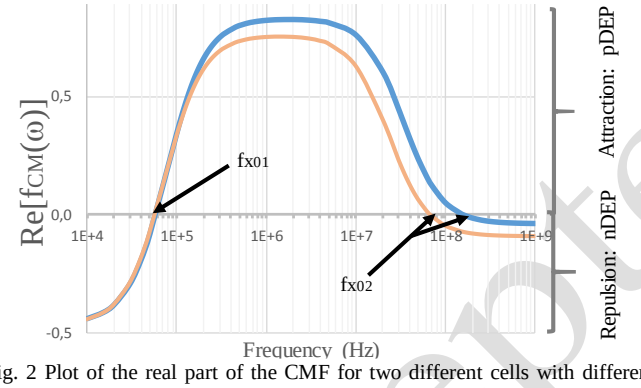

Fig. 2 Plot of the real part of the CMF for two different cells with different cytoplasm dielectric properties.

Consequently, the CMF is frequency dependent and will differ from one cell type to another according to the dielectric permittivity and conductivity specificities of their cytoplasm. As illustrated in Fig.2, this property can be exploited to discriminate and sort cells especially for frequencies above the MHz range where intracellular features predominate. One can also notice that the sign of $Re[f_{CM}(\omega)]$ influences the type of DEP force applied to cells. For positive CMF, cells are attracted by the E field: usually we talk about positive DEP (pDEP). Whereas for negative CMF, they are repealed away (nDEP). The CMF spectral plot lets also appear two crossover frequencies (f_{x01} & f_{x02}) for which F_{DEP} becomes zero, so has no influence on the cell trajectory.

In the proposed cytometer, cells are flowing in microfluidic channels (Fig.1). Then they are subjected to a hydrodynamic flow and E field effect. As a result, each cell trajectory depends on a combination of both hydrodynamic and DEP forces. So, setting the DEP signal frequency close to f_{x02} presents an interest, because it will allow to tune and finely adjust the resulting cell displacement under combined E field and flow influence.

Fig.3 illustrates how can react a cell entering in the electric field generated by two thin electrodes patterned in a microfluidic channel. The fluid flow drives the cell from the right to the left. Actually, this is the force balance that determines if a cell can pass or not through the electric field generated by the electrodes area. In cases for which nDEP (Fig.3 (c)) and pDEP (Fig.3 (b)) forces are weak, the hydrodynamic forces are stronger than the DEP force, and the cell continues to travel in the channel with a more or less strong deviation or altitude change [7].

In the case for which the DEP force is greater than the hydrodynamic one, the electric field stops the cell motion. Hence, under strong nDEP influence (Fig.3 (d)), the cell is forcefully repealed from electrode gap to remain at an equilibrium position where a balance between the two forces is reached. The E field acts here as a wall that prevents cells to cross high field area. To the contrary submitted to strong pDEP force (Fig.3 (a)), the cell is attracted and deviated until reaching the strongest E field intensity zone where it may stay trapped. Actually, this zone is located in the gap between electrodes, where an undesirable cell adhesion on the substrate or even on the electrodes can occur.

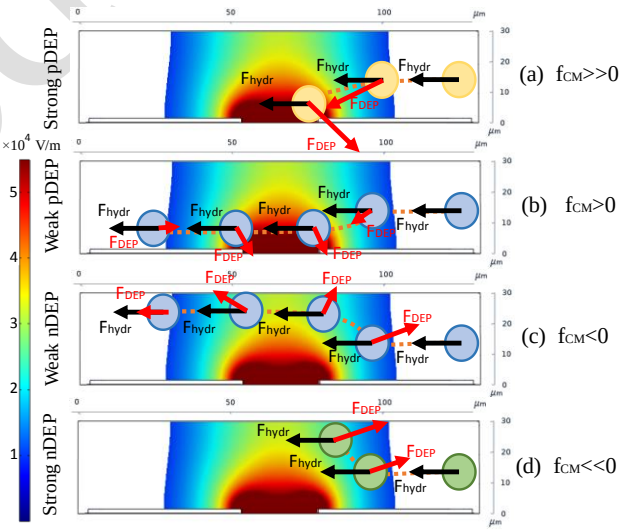

Fig.3 Electric field influence on the flowing cell trajectory related to its CMF value. COMSOL Multiphysics simulation for 2V applied signal.

B. Proper DEP frequency choice for efficient sorting

For envisioned applications, a continuous flow is generally preferable for the cell suspension to be treated especially if a high throughput cell sorting is targeted. For such purpose, cell trapping during the sorting process has to be avoided; since it may favor cell agglomerate formation that disturbs the flow and may change sorting efficiency or even generates microchannel clogging and results in the cytometer failure.

To set a DEP signal able to achieve efficient cell sorting, we recommend to select a frequency higher than or at least close to the f_{x02} of the cell population of interest. Indeed as illustrated in Fig.4, as two cell types are considered with existing spectral

CMF differences, it is possible to separate the two populations by setting the cytometer frequency just above the highest median crossover frequency value between both cell types ($f_{x02B\ Median}$ here).

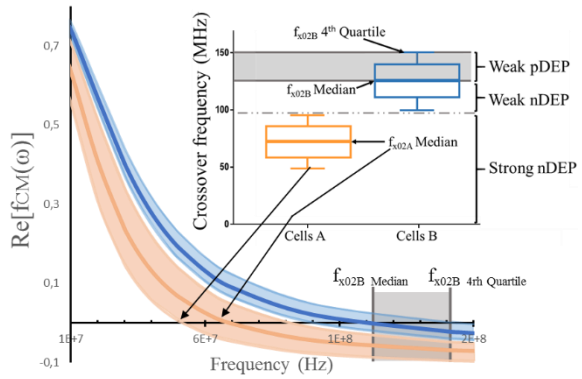

Fig. 4 Plot of CMF and dispersion of fx02 for two different cells types. The grey frequency range corresponds to proper DEP frequencies.

Natural cell heterogeneity occurring inside a whole population implies difference of dielectric feature between cells and results in crossover frequency dispersion [4]-[8]. Taking into account such dispersion for f_{x02A} & f_{x02B} frequencies, one can see that all type A cells should be submitted to strong nDEP forces resulting in a strong repulsion by the E field. Whereas most of type B cells might only see very weak forces that result in a limited trajectory deviation. As shown on Fig 4, suitable DEP sorting frequencies can be selected in upper range of cell B crossover frequencies while paying attention to limit the probability of pDEP cell trapping. Actually the sorting process should be all the more efficient that differences of crossover frequency between cell populations will be large with a restricted frequency overlap between extreme values.

C. *Cytometer design*

The proposed cytometer design is based on pair of gold electrodes separated with a 23µm wide gap in order to generate a localized high intensity E field barrier. With this configuration, as illustrated in Fig.5, the strongest part of the electric field is concentrated in this gap. The narrower electrode, appearing in Fig.5, is 35µm wide and 2mm long and connected to the center conductor of a 50Ω CPW line used to properly bias the cytometer at UHF frequencies. The other electrode is grounded. Following similar principle than [9], these electrodes have been set with a 20° angle related to the main flow direction, in order to induce an efficient cell trajectory deviation under strong nDEP conditions. Indeed, with such electrode design it is expected to fully deviate the cell from the bottom to the top side of the sorting zone (Fig.7 (a)). This angle bas been chosen to optimize the required E field intensity for a targeted range of flowing cell velocity. Indeed, a wider angle would imply generating stronger DEP force to reach similar cell trajectory with the same flow velocity. Whereas a softer angle would require to extend the sorting zone size that results in a less compact cytometer design.

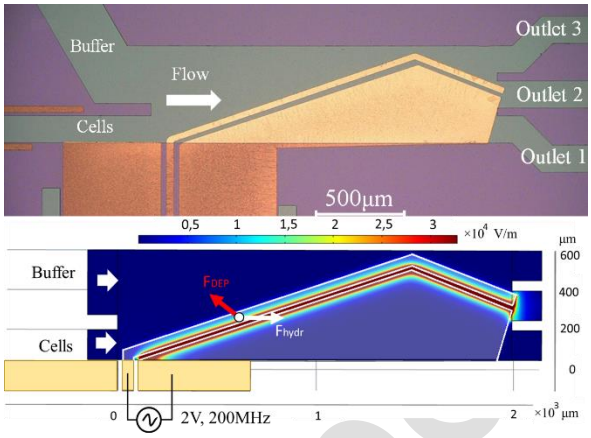

Fig. 5 Top view of the cytometer main sorting area and associated E field distribution plot. The electrodes have been drawn clearer with white edge.

The microfluidic network of the cytometer includes two main inlets from which the cell suspension and a buffer solution are injected. Balancing the injection flow between each inlet allows adjusting the point where the cell trajectory should cross the E field barrier and initiate its possible deflection. Three outlets has been also considered in order to potentially isolate different cell subpopulations. The center outlet (n°2) is expected to collect cells having undergone negligible trajectory deviation. Whereas cells submitted to strong nDEP influence should be driven to outlet 3, and outlet 1 should collect cells subjected to a weak nDEP forces that made them jumping above the E field barrier (Fig.3 (c)) and repealed away above grounded electrode surface.

III. Experimental Results

A. *Materials and Methods*

The presented cytometer has been fabricated on high resistivity silicon wafer covered by a 1µm thick silicon oxide. 2µm thick gold layer was used to pattern the electrodes. The microfluidics channels have been molded in a PDMS cover which has been aligned above the electrodes and stacked on the silicon chip forming a 38µm high microchannel network.

The cells used during our experiments were Mesenchymal Stem Cells (MSC) routinely cultured. The culture was realized in a F12 DMEM medium, supplemented by 10% FCS and 20ng/mL of bFGF (basic Fibroblste Growth Factor) at 37°C in a humidified 5% CO_2-95% air incubator. They are been seeded at 5×10^5 viable cells/ml in appropriate flasks.

Cells were suspended in dedicated DEP sucrose medium presenting a conductivity of 22,4mS/m. To maintain cells alive during experiment, the medium osmolality has been set at 304mOsm by adjusting the sucrose concentration in pH buffered deionized water.

B. *Results*

Based on using a similar measurement principle than used in [7-9], used mesenchymal cell cultures had been beforehand characterized considering the same suspension DEP medium for all our experiments. The expected range of frequencies, for which cell crossover should occur, can be seen from data

collected on 21 characterized cells and summarized in Fig.6. Hence, the heterogeneity of the cell culture translates into a statistical representative dispersion of f_{x02} ranging from 75 to 160MHz with a median crossover at 90MHz

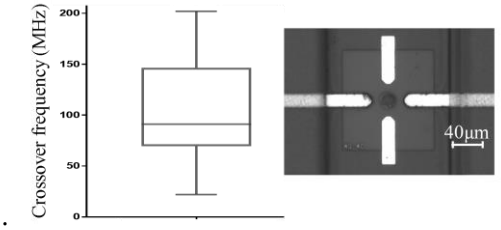

Fig. 6 Range of crossover frequencies for cultured mesenchymal cells.

Fig.7 shows typical cell ratio collected at the different cytometer outlets for various applied DEP signal frequencies. As expected, at 200MHz almost all cells react strongly in nDEP and are deflected to the top outlet (Fig.7 (a)).

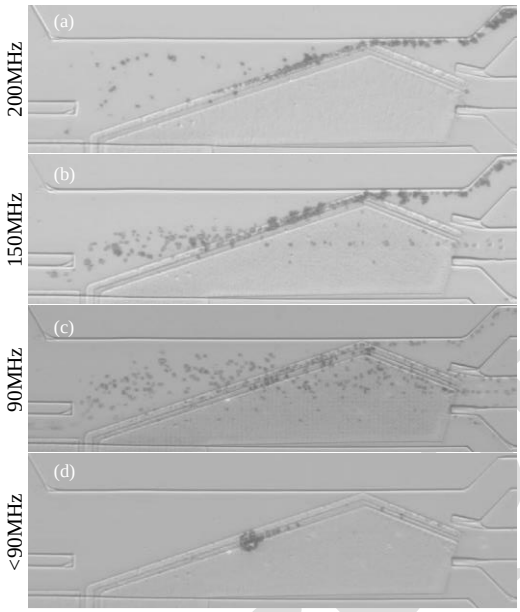

Fig. 7 Influence of the DEP frequency on MSC cell subpopulation separation.

If the frequency is decreased to 150MHz corresponding to the third quartile of Fig.6, we can observe that an increasing number of cells are not deflected anymore and now collected at the center outlet (Fig.7 (b)). This result makes sense since roughly 25% of MSCs should be submitted to very weak DEP forces. At 90MHz, the cell ratio that reaches the central and bottom outlets strongly increases (Fig.7 (c)) especially because the DEP frequency becomes closer to the median crossover frequency of the cell population. For frequencies lower than 90MHz, many cell trapping occurs, related to a too large number of cells that now react in pDEP. As a result, some cell aggregates start to be formed (Fig.7 (d)). However, this phenomenon can be triggered adjusting the intensity of the E field or the flow velocity at the expense of the cytometer sorting efficiency: some tradeoffs can be found to operate at such frequencies.

IV. Conclusion

This work shows that biological cells can be successfully separated related to their intracellular dielectric specificities using dielectrophoresis at UHF frequencies. A microfluidic cytometer chip has been designed aiming to efficiently combine hydrofluidic and repulsive DEP forces with different strength related to own cell features. It should allow sorting cells based on their difference of crossover frequencies. For present experiments, it has been confirmed that setting properly the DEP working frequency, different cell subpopulations can be separated in mesenchymal cell cultures. These results are in good agreement with the observed heterogeneity of crossover frequencies for this cell culture. Others experiments are currently is progress, as sorting mixture of different cell types assisted by fluorescence imaging control. Latest results will be presented during the conference.

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