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# Ultra-High Frequency Dielectrophoresis to Characterize Mesenchymal Stem Cells Differentiation: Application to bioceramics synthesis

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**Abstract** — This article aims to investigate the Ultra-High Frequency Dielectrophoresis technique to characterize populations of human mesenchymal stem cells. The principle is based on studying the displacement of a cell in an electric field gradient to quantify a characteristic frequency that establishes the UHF dielectrophoretic 'signature' of the investigated cell population. Preliminary results obtained from patient-derived cultures, in correlation with conventional biological analyses, seem to indicate that the osteogenic differentiation of mesenchymal stem cells can be detected by UHF-DEP, making this technique an interesting approach to characterize hMSC differentiation in the context of optimizing bioceramics for bone regenerative medicine.

**Keywords** — dielectrophoresis, ultra-high frequency, human mesenchymal stem cells, osteogenic differentiation.

## I. INTRODUCTION

The treatment of significant bone defects requires the use of natural or synthetic bone grafts. Calcium phosphate-based ceramics (CPC), such as hydroxyapatite, exhibit excellent biocompatibility, making them a commonly used biomaterial for bone repair (eg [1]), but not entirely satisfactory (eg [2]) for large defects. The development of bioceramics with optimized biological properties, such as the stimulation of host mesenchymal stem cell (MSC) differentiation into mature bone-forming cells, requires a detailed understanding of the biomaterial/live tissue interface, which depends on its surface chemical and physical properties. The introduction of chemical species into the crystalline lattice of CPC is one of the strategies frequently followed. Whether the interaction of cells with the tested material and its dissolution products released in biological milieu enhances or not the kinetics and extent of osteogenic differentiation must be then determined. This process is commonly evidenced in cells through the evaluation of gene and protein expression of markers (eg RUNX2, Type I Collagen) and the production of mineralized extracellular matrix. These biological assays, that aim to evaluate the properties of biomaterials, are time-consuming and costly. Indeed, targeting panels of markers is necessary, which involves the use of a large number of samples to obtain reliable information. A method able to detect cell differentiation using a minimal number of samples would be of high interest for optimization of chemical composition of biomaterials by predicting the optimal amount of doping species.

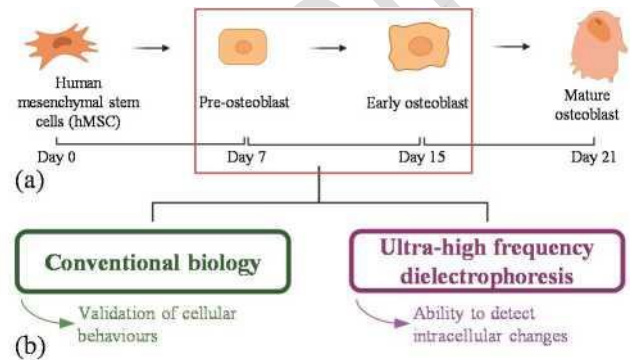


Fig. 1. (a) Osteogenic differentiation process and (b) schematic illustration of the used methodology for cell differentiation characterization.

In this context, the objective of this article is to demonstrate the feasibility of characterizing the differentiation state of human mesenchymal stem cells (hMSCs) (eg Fig. 1a) using an innovative diagnostic tool based on a label-free electromagnetic (EM) microsensor. Indeed, hMSCs are multipotent stem cells that are involved in bone tissue repair and are capable of differentiating into osteoblasts, bone-forming cells (eg [3]).

As illustrated in Fig. 1.b, the methodology followed will involve correlating the cellular differentiation state (eg Fig. 1a) monitored by conventional biology approaches, with the electromagnetic properties of the cells characterized by Ultra-High Frequency Dielectrophoresis (UHF-DEP), referencing non-induced cells (MSC). The UHF-DEP principle allows non-invasive characterizations of the intracellular content of cells. For this purpose, hMSC are cultured in a medium containing or not osteogenic differentiation inducers and analyzed at different time points.

## II. MATERIAL AND METHODS

### A. Ultra-High Frequency Dielectrophoresis: opportunities

Biological cells are polarizable particles that, under the influence of a non-uniform electric field, experience forces that allow them to be manipulated. This force, known as dielectrophoresis force ( $F_{DEP}$ ), relies on the dielectric properties (conductivity and permittivity) of the cell, related to the

properties of its suspension medium, as shown by equations (1) and (2).

$$F_{DEP} = 2n \tau \operatorname{Re}[f_{cm}(M)] V E_{rms} \quad (1)$$

$$\frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2} \quad (2)$$

suspension medium,  $f_{cm}$  is the Clausius-Mossotti factor,  $E$  is the applied electric field (considered in root mean square value), Here,  $r$  is the radius of the cell,  $S_m$  is the permittivity of the and  $E^*_{,m}$  respectively represents the complex permittivity of the cell and the hosting medium.

Fig. 2 illustrates the frequency dependence of the real part of the Clausius-Mossotti factor. If the real part of the Clausius-Mossotti factor is positive, then cells are subjected to an attractive force, which is called positive DEP (pDEP). Otherwise, if the real part of the Clausius-Mossotti factor is negative, the force is repulsive, corresponding to the negative DEP (nDEP).

We can notice two singular frequencies, referred to as transition frequencies and respectively noted as  $f_{x01}$  at low frequencies (LF) and  $f_{x02}$  at high frequencies (HF), for which the real part of  $f_{cm}$  equals zero, thus generating a  $F_{DEP}=0$ . In [5], Ronald Pethig demonstrated that depending on the frequency used for the dielectrophoresis signal, the cellular properties involved differ. As illustrated in Fig. 2, at low frequencies, the influencing parameters are rather related to the morphology, size, and membrane properties of cells.

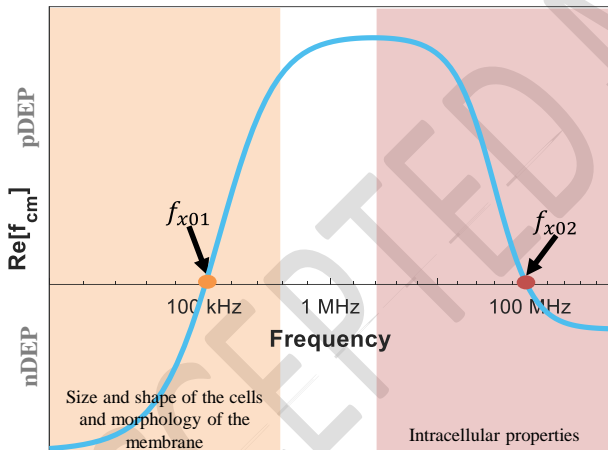


Fig. 2. Frequency dependence of the Clausius-Mossotti factor.

At higher frequencies, where  $f_{x02}$  is located, the intracellular content and its overall dielectric properties dominate. This last point has been experimentally demonstrated in previous works, where ultra-high frequency dielectrophoresis has been used to characterize and discriminate cancer stem cells by probing their intracellular contents (eg [6], [7]).

### B. Ultra-High Frequency Dielectrophoresis: experimental set-up

As illustrated in Fig. 3, the UHF-DEP signal applied to the cells is generated by a high-frequency source (R&S® SMB100A) coupled with a wideband amplifier (Bonn Elektronik BLWA 110-5M), able to produce an amplified signal

with a 40 dB gain. The signal is applied to the EM sensor using RF probes (MPI TITAN T26P-GSG-150) touching RF CPW lines matched to 50 Ohms.

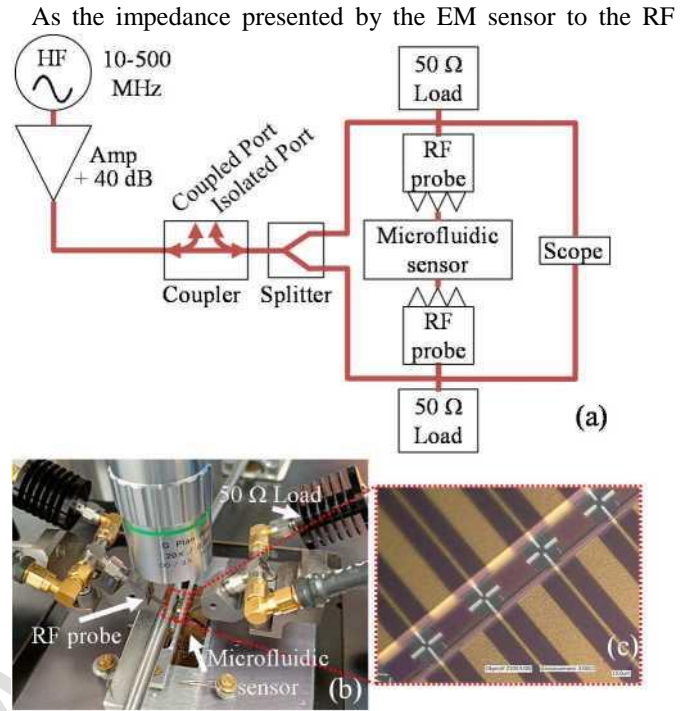


Fig. 3. (a) Schematic diagram of the characterization setup; (b) Photograph of the EM sensor under the probe and (c) Zoom on the sensors implemented in the microfluidic channel.

probes is very high, a 50 Q load is added in parallel with each probe to ensure proper impedance matching to the amplifier and the delivery of a controlled voltage magnitude signal regardless of the frequency. The applied voltage on the electrodes is monitored using an oscilloscope (Tektronix DPO4104) to control the quality of the generated waveform.

The UHF-DEP microsensors principle and the measurement protocol were extensively described in [6]. The design is based on four electrodes that allow the generation of an electric field gradient as illustrated in Fig 4.

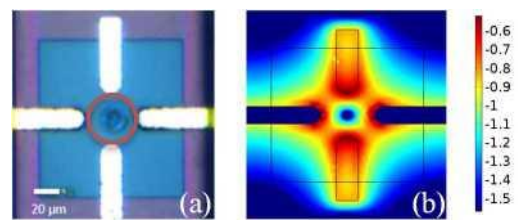


Fig. 4. (a) Zoom on one of the sensors implemented in the microfluidic channel and (b) Computation of the biased sensor in a non-uniform electric field (COMSOL Multiphysics®). The scale color corresponds to the normalized electromagnetic field intensity (eg [7]).

A microfluidic channel has been integrated above the EM microsensors, to allow the manipulation of the cells flowing toward the center of the microsensors (red circle in Fig. 4a) and to enable flow adjustment to stop the cell before analysis.

Indeed, when a cell approaches the center of the sensor, the flow is stopped, and the electrodes are biased with negative DEP

signal to trap the cell at the center of the quadrupole, where the electric field is the weakest (blue weak field areas in Fig. 4b). The DEP signal frequency is then progressively reduced until the cell switches to positive DEP, moving towards the edge of one of the lateral electrodes where the E field is strong (red area in Fig. 4b). The DEP signal frequency initiating this cell movement corresponds to the HF transition frequency  $f_{x02}$ . This measurement is repeated on approximately 40 different cells per characterization campaign and repeated three times for each cell culture condition. This data compilation will lead to the UHF-DEP signature of the characterized cell population. Mann-Whitney statistical tests were used to investigate the significance of the results obtained, with p-values < 0.05 considered significant.

### C. Cell culture

hMSCs are isolated from adipose tissue in surgical waste collected at the Maxillofacial Surgery Department of Limoges University Hospital (LUH) with authorization from the LUH Ethics Committee (authorization No. 312-2019-78, 12/09/2019). The cells are registered at the Biological Resource Center of LUH in compliance with regulatory requirements, under declaration CODECOH No. DC-2008-604. To account for cellular heterogeneity, cells isolated from two different patients (named Patients A and B in this article) were analyzed.

The cells are seeded at a density of 5000 cells/cm<sup>2</sup> and cultured during 7 to 15 days (time needed to obtain pre-osteoblasts or early osteoblasts) either in a growth medium (GM) composed of  $\alpha$ -MEM without ascorbic acid supplemented with 10% fetal bovine serum and antibiotics (50 IU/ml penicillin and 50 pg/ml streptomycin), or in osteogenic differentiation medium (ODM) composed of GM containing 100 nM dexamethasone, 50 pg/ml ascorbic acid, and 10 mM  $\beta$ -glycerophosphate (osteogenic inducers) [4]. The cultures were maintained at 37°C in a humidified air atmosphere containing 5% CO<sub>2</sub>.

Before UHF-DEP characterizations, hMSCs are detached from their substrate. Due to the normal process of extracellular matrix maturation (mineralization), the cells are more resistant to detachment. For this reason, cell detachment is performed by incubating the cells in EDTA (5 mM) for 10 minutes to chelate calcium and in collagenase type 1 (0.2%) for 5 minutes to digest the extracellular matrix.

They are then centrifugated at 300g for 5 minutes and suspended in a low-conductivity osmotic medium, well-suited for DEP experiments. This medium is composed of deionized water and sucrose. Its conductivity is 21 mS/m with a pH of 7.6.

The UHF-DEP signature differences that may be observed primarily rely on intracellular features, then to correlate the UHF-DEP characterizations with biological indicators, fluorescence labeling analyses have been performed to image the main intracellular changes in cell culture. For that, cells were previously seeded onto borosilicate glass slides under the same culture conditions, rinsed in PBS 1X before fixation in 4% paraformaldehyde in PBS 1X, then permeabilized for 10 min in Triton X-100 0.1%. Aspecific antigenic sites were blocked in PBS bovine serum albumin 3% for 30 min. Nuclei were labeled by a 5 min immersion in Hoechst 33342 20 pM. Type 1 collagen was immunolabeled by first hybridization with anti-collagen 1 antibody (PA5-95137, ThermoFisher) followed by incubation

with goat-anti rabbit antibody conjugated to AlexaFluor 594. Microscopic examination was performed using an Axioimager M2 fluorescence microscope (Zeiss).

## III. RESULTS

Measurement campaigns were achieved to observe the differences in biological and dielectrophoretic properties between hMSC cultured in the growth medium and in the osteogenic differentiation medium for 7 and 15 consecutive days. For each condition, biological tests to validate the cellular differentiation state vs DEP-UHF characterizations were performed in three independent experiments for both patients.

### A. Comparison of cell features and marker expressions

To characterize the initial stages of osteogenic differentiation, we focused on collagen I, a protein involved in the formation of bone tissue. Fig. 5 shows fluorescence images where cell nuclei appear in blue and type I collagen in red. A distinctive expression of collagen I is observed in ODM, consistent with osteogenic differentiation.

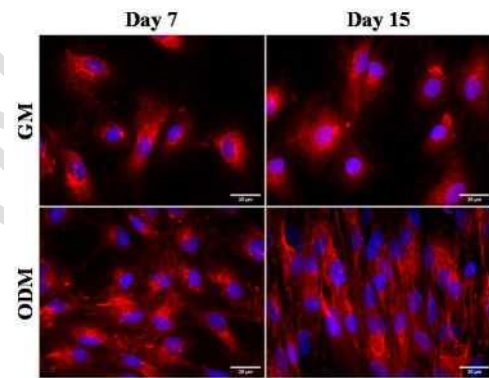


Fig. 5. Tracking of the evolution of stem cells cultured in GM or in ODM over time using fluorescence microscopy. (Blue: nuclei, red: type I collagen)

After 7 days of culture, analysis of fluorescence images highlighted that the cell density is higher in the differentiation medium. Furthermore, we observe increased cell overlap and a decrease in the average surface area and roundness of the nuclei compared to cells cultured in the growth medium. Distinctive variations in the distribution pattern of collagen I expression are also evident between the GM and ODM conditions. These results underscore significant morphological differences as well as modulation of collagen I expression, validating the engagement of cells cultured in ODM along the osteogenic differentiation pathway. Despite the natural heterogeneity of the cells, it is noteworthy that the results obtained are identical for both patients.

### B. UHF-DEP characterizations

To establish a correlation between the physical and biological results, the HF transition frequencies ( $f_{x02}$ ) of cells from the two patients were characterized through several measurement campaigns and under different conditions: based on cumulative culture times (7 and 15 days) and the culture medium used (GM and ODM). All collected experimental data are summarized in Fig. 6.



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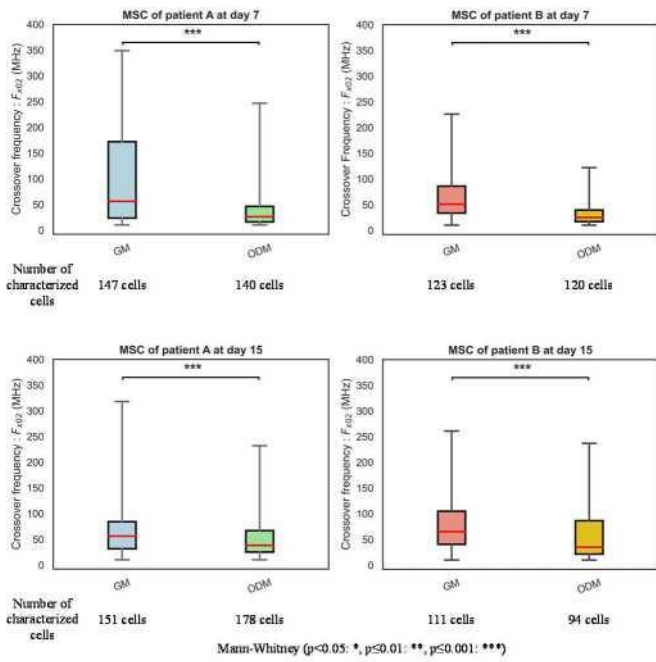


Fig. 6. Boxplot representing the measured transition frequencies of stem cells in different conditions (7-15 days / GM-ODM).

The frequency distributions of measured transition frequencies are presented in the form of boxplots. The red line matches with the median value of the signature, with 50% of individuals falling on either side of this value. The dispersion of transition frequencies measured for different populations under various culture conditions can be attributed to the inherent biological heterogeneity present in primary cultures of stem cells.

Overall, the values of  $f_{c02}$  measured for cells cultured in ODM, thus engaged to the osteogenic differentiation pathway, are significantly lower than those measured for cells cultured in growth medium for which the stemness is maintained. Characterizations using conventional methods of biological analysis of the physiological state of these stem cells indeed validate the presence of pre-osteoblastic cells at 7 days in ODM and hMSCs in GM.

## IV. CONCLUSION

To the best of our knowledge, this is the first demonstration of the use of UHF-DEP to characterize mesenchymal stem cells and their differentiation in the osteoblastic pathway. These preliminary results seem to indicate that the differentiation of MSCs into osteogenic cells induces a significant decrease in the measured HF transition frequencies, demonstrating the potentiality of the UHF-DEP approach for characterizing mesenchymal stem cell differentiation.