Electrophysiological Characterization of Mesenchymal Stem Cells via Dielectrophoresis

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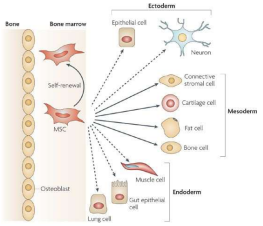
ABSTRACT

Stem cells play an important role in regenerative medicine because of their ability to proliferate into various cell types. This work focuses on murine mesenchymal stem cells (MSCs) differentiating into tenogenically differentiated cells, over the course of their differentiation on treatment with certain growth factors, MSCs are observed to exhibit different phenotypical and genotypical changes accounting for their change in electrophysiological make-up, i.e., the capacitance and permittivity. In this study, murine MSCs are characterized using dielectrophoresis (DEP), a non-labeling, non-destructive electrokinetic technique using crossover frequency method. DEP is known to be able to detect subtle changes within the cell. Studies were completed with untreated murine MSCs, and murine MSCs treated with transforming growth factor β(TGFβ)2 to initiate differentiation towards tendon; the treated MSCs were treated on a time scale of 0 day, 3 days, and 7 days. The results showed that tenogenically modified MSCs could be distinguished from the untreated control group MSCs as early as 3 days into treatment.

INTRODUCTION

MESENCHYMAL STEM CELLS

Mesenchymal stem cells are stem cells found in bone marrow & can differentiate into a variety of cell types, including bone cells, cartilage cells, muscle cells, and fat cells.



DIELECTROPHORESIS (DEP)

- Non-uniform electric field induces motion in polarizable particles, which is the termed as DEP.
- Motion of the dielectric particles towards high intensity field and low intensity fields are termed as n-dep and p-dep respectively.
- The frequency at which the net force acting is zero is termed as Dielectrophoretic crossover frequency.
- DEP is a label free and high throughput technique and is known to determine subtle morphological changes within biological entities.

GOAL: To determine the electrophysiological properties of murine mesenchymal stem cells using dielectrophoresis.

MOTIVATION: To develop a microfluidic sorting platform to effectively separate tenogenically differentiated stem cells for use in stem cell regenerative medicine.

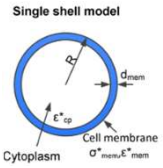
SINGLE-SHELL MODEL:

$$f_c^* = \frac{1}{2\pi}\left\{\frac{-[(1-A_{0p})\sigma_m + A_{0p}\sigma_p](\sigma_m - \sigma_p)}{[(1-A_{0p})\varepsilon_m + A_{0p}\varepsilon_p](\varepsilon_m - \varepsilon_p)}\right\}^{\frac{1}{2}}$$

$$\sigma_p = \frac{br}{a}\cdot G_{s\_mem}$$

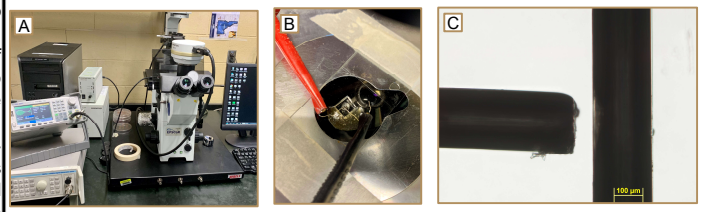
$$a = \frac{1-A_{0p}}{A_{0p}} \qquad b = \frac{c}{2c+r}$$

$$r\cdot f_c = \frac{A_{0p}}{2\pi b C_{s\_mem}}\left\{\frac{\left(\left(\sigma_m - \frac{br}{A_{0p}}G_{s\_mem}\right)\times\right)}{\left[\left(\frac{1-A_{0p}}{A_{0p}}\right)\sigma_m - \frac{br}{A_{0p}}G_{s\_mem}\right]}\right\}^{\frac{1}{2}}$$



f_c = cross frequency obtained for a single shelled spheroids of radially-symmetric radius r rotating about a normal minor axis, c. ε and σ are the real permittivity and conductivity with the subscripts m and p representing medium and particle respectively. C and G are capacitance and conductance with the subscript s_mem denoting per unit area of particle. A_{0p} is given as 0.236.

EXPERIMENTAL SETUP



A. Experimental Setup
B. Microwell Device on Microscope Stage
C. Electrode Spacing - 75 µm

Frequency was swept at a fixed amplitude of 8 Vpp

Medium Property	Electrode Spacing	Suspension	Frequency Range
50 g/L Dextrose Conductivity: 0.06 S/m	< 100 µm	~ 10^6 cells/ml	10 kHz – 80 MHz

OBSERVATIONS


Fig 1: Baseline – N-DEP

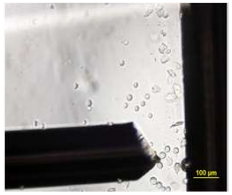


Fig 2: Baseline – P-DEP

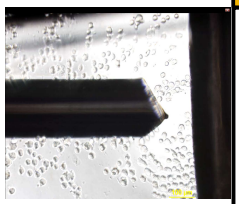


Fig 3: 0 day untreated – N-DEP


Fig 4: 0 day treated – P-DEP

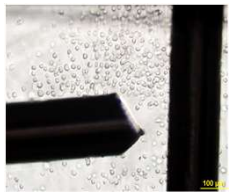


Fig 5: 3 day untreated – N-DEP


Fig 6: 3 day treated – P-DEP

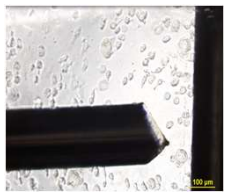


Fig 6: 7 day untreated – N-DEP


Fig 7: 7 day treated – P-DEP

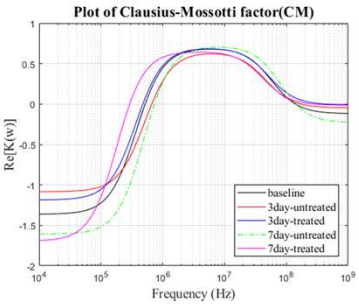
- Dielectrophoretic experiments were performed by suspending 2µL of the prepared medium until crossover frequency was determined.
- Experiments were performed on 0, 3 and 7 day time points. Experiments were ran 3 times each time point to check the consistency of the determined frequencies.
- Experiments were also performed on different devices to determine if variability of device affects results.
- It was noticed that the variation is ± 3%.

RESULTS

Properties	Baseline Cell
Total Membrane Capacitance	8.4853 pF
Permittivity	15.2524

Properties	3-Day Untreated	3-Day Treated
Total Membrane Capacitance	6.6813 pF	9.4281 pF
Permittivity	12.0097	16.9471

Properties	7-Day Untreated	7-Day Treated
Total Membrane Capacitance	5.9756 pF	14.142 pF
Permittivity	10.7411	25.4206



DISCUSSION & CONCLUSIONS

- The dielectric properties of MSCs vary significantly for different groups. Using this difference in properties, tenogenically differentiated cells can be sorted from the untreated controls as early as 3 days of treatment.
- TGFβ2 which is used to initiate the differentiation, affects cell dielectric properties significantly.
- The 0 day treated and untreated MSC's displayed aberrance and aren't significantly different from each other and separation of these may not be effective. This indicated TGFβ2 –induced tenogenesis may require at least 24 hours to change the dielectric properties.

FUTURE WORK

- To develop a microfluidic sorting platform to separate stem cells of different time points using dielectric properties.
- Modelling of microfluidic sorting platform using COMSOL to optimize sensitivity and specificity.
- Validation and optimization of the sorting platform for different treatment groups, as early 72 hours will be analyzed.

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