

Dielectrophoretic Fractionalization of Erythrocytes at Varying Osmolarities

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Introduction

Dielectrophoresis (DEP) is a method of inducing forces on particles by subjecting them to an alternating electric field. At relatively high frequencies cells tend to be attracted to the electrode surface and trapped, while at low frequencies cells tend to be repulsed. The unique frequency at which cells make this transition from being attracted to repulsed is called the crossover frequency, and is dependant on a particular cells' dielectric parameters.

Our experimental apparatus utilizes a long shallow chamber through which cell suspensions are pumped at low velocities. Taking advantage of the resulting velocity profile, a strategy known as field flow fractionalization (FFF), cells lifted to varying heights with DEP will elute from the chamber at different times. Their size and elution time are then recorded using a flow cytometer or particle counter.

The premise of this experiment was to see if the physical changes in erythrocytes, or red blood cells, could be observed by our DEP-FFF assembly for varying osmolarities of buffer solution. As the osmolarity of the buffer decreases, water molecules diffuse through the cell membrane and inflate the cells from their characteristic shape to a more spherical appearance.

Methods

Approximately 10⁶ cells were injected into the chamber and allowed to settle for 8 to 10 minutes before a flow of 6 to 10 mL/min is initialized for each run. At each osmolarity of solution being tested, four runs were subjected to various electrical signals to characterize the cells moving through the chamber:

- **No signal** gives general information
- **15 kHz signal** lifts most cells and debris and yields information on cell density
- **300 kHz to 15 kHz sweep signal** lifts and elutes cells according to their crossover frequency
- **300 seconds at 300 kHz, then 15 kHz signal** is used to qualify cell movement while depressed

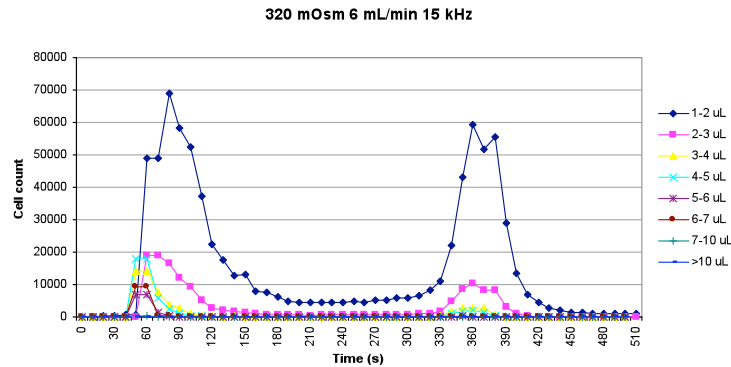


Fig. 1 Sample particle counter output

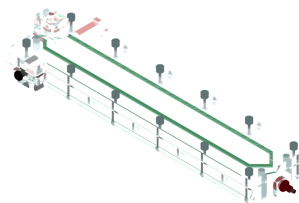


Fig. 2 DEP-FFF chamber

Results

The results from each run were plotted in a format similar to Figure 1 and analyzed to find the peak time of arrival.

These arrival times are listed in Table 1, along with the duration of each event. Table 2 shows that cell density decreases linearly with decreasing osmolarity, while the crossover frequencies increase nonlinearly.

Table 1

Experimental Results				
Signal mOsm	No Field	15kHz	300kHz for 300s, then 15kHz	
	Peak (s)	Distribution (s)	Peak (s)	Distribution (s)
320	1300	1000	370	150
250	1020	700	360	140
180	850	600	310	150
120	900	700	280	130
Signal mOsm	Logarithmic Sweep	300kHz for 300s, then 15kHz		
	Peak (s)	Distribution (s)	Peak (s)	Distribution (s)
320	610	200	640	150
250	590	200	605	150
180	520	160	570	120
120	470	150	550	100

Table 2

Analytical Results		
mOsm	Cell density	Crossover frequency
320	1094.50	138.36
250	1063.91	145.45
180	1068.81	160.72
120	1055.88	177.60

Analysis

Erythrocyte density

An analysis completed recently¹ showed that relative cell density could be determined from DEP-FFF elution profiles at 15 kHz. It was shown that the calibration between elution time and density is approximately linear. Using this mapping, the theoretical relationship between the cell density and osmolarity

$$\rho = \frac{O_s m}{O_{s0} V_0}$$

is empirically validated in Figure 3 with a reasonably linear relationship.

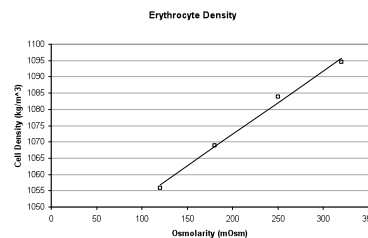


Fig. 3 Plot of erythrocyte density estimated from DEP-FFF elution at 15 kHz versus osmolarity

DEP-FFF crossover frequency

The length of time it takes for the cells to lift and elute with the sweep signal can be used to deduce the crossover frequency f_{co} . Plotting the crossover frequencies against $O_s^{-1/3}$ reveals that the results attained with the DEP-FFF process agree well with the predicted osmolarity model

$$f_{co} = \frac{\sigma_s}{\sqrt{2\pi} r_0 C_0} \left(\frac{O_{s0}}{O_s} \right)^{1/3}$$

as seen in Figure 4.

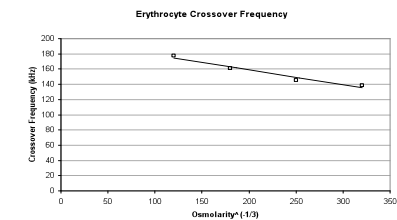


Fig. 4 Variation of crossover frequency with osmolarity

Conclusions

The densities deduced by the DEP-FFF procedure decreased proportionately with decreased osmolarity, which is consistent with the premise that erythrocyte volume is proportional to the osmolarity of the suspension. Additionally, the change in dielectrophoretic crossover frequency was found to be proportional to the cubic root of the osmolarity as predicted. These results indicate that the DEP-FFF model is versatile enough to account for and detect the effects of varying osmolarity in cell suspensions, and has the potential to be further developed into a robust means of characterizing the biophysical properties of cells.

References

1. Cell dielectric, density and deformability profiles from dielectrophoretic-field flow fractionation, submitted Biophysical Journal June 28 2009

Figure 1 was generated using a a student license of Autodesk Inventor Software

