**Analysis of Microvascular Endothelial Cell Morphology During Migration on Haptotactic Fibronectin Gradients**

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**Statement of Purpose:**

Cell migration is known to be a key component of tissue integration, vascularization, wound healing, and biocompatibility.[1] Recent work has characterized cell migration on both uniform concentrations of cell adhesion molecules as well as haptotactic gradients. Although directed migration towards higher concentrations of adhesion ligand has been observed and drift speed has been shown to increase with gradient slope, no clear cellular mechanism for this process has been suggested.[2] Due to the variability of cell behavior during lengthy migration experiments it is difficult to interrogate intracellular processes with time averaged migration parameters. To understand this process more clearly investigations need to move towards correlations of instantaneous migration parameters and observed cell properties. To begin to address this issue, this study investigates the relationship between cell morphology and the discrete calculated drift speed and angular volatility during migration of microvascular endothelial cells on haptotactic fibronectin gradients.

**Methods:**

Methods for cell culture and gradient generation have been described previously.[2] Cell migration was monitored on the surface of the fibronectin gradients for 24-48 hours by videomicroscopy on the stage of a Zeiss Axiovert S100 microscope. Cell positions were recorded at 15-minute intervals and fit to the Langevin model shown below using a maximum likelihood estimation algorithm.[2]

$$\frac{d\vec{v}}{dt} = -\beta\vec{v} + \sigma\vec{W}(t) + \alpha\hat{x}. \qquad \text{Eq 1.}$$

Angular volatility is defined as the relative change in the instantaneous directional unit vector at discrete locations along the continuous calculated cell trajectory in a one-hour window. Cellular drift speed along the gradient is defined as $\alpha/\beta$ from the Langevin fit. Cell morphology values were measured manually. The gradient polarization ratio is defined as the length of the cell along the gradient direction divided by the length of the cell on the perpendicular axis. The cell polarization ratio is defined as the length of the cell on its longest axis divided by the length on the perpendicular axis. A local regression (S-Plus f(loess)) with a span of 0.5 was conducted on the instantaneous drift speed data to determine speed variations on the timescale of changes in the cell polarization data.

**Results / Discussion:**

The variablilty of cell morphology during migration experiments makes analysis of discrete polarization and speed values essential to characterize this relationship. Figure 1 presents a distribution of de-trended cellular drift speeds as a function of gradient polarization ratio. The line represents a linear fit with a $R^2$ value of 0.75. The correlation coefficient for this data set is 0.87. This strong correlation demonstrates the computational power of instantaneous measurement for studying cell migration behavior.

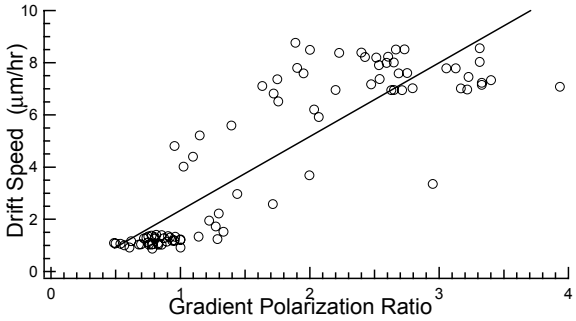


**Figure 1**. Cellular drift speed is highly dependent on the Gradient Polarization ratio.

Figure 2 presents a comparison of cell polarization ratios with high and low angular volatility. Cells with high angular volatility have statistically lower polarization in both cases ($p<.05$). This data compliments the drift speed data by implying that cells that are consistently moving in one direction are more polarized. Cells that are in the process of turning appear to go through a rounding process and become less polarized.

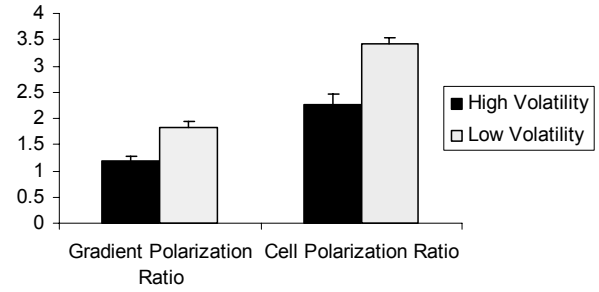


**Figure 2**. Cells with high angular volatility have lower observed polarization ratios.

**Conclusions:**

This study demonstrates the utility of correlating computational analysis of cell migration tracks with discrete measurements of cell properties. Cell polarization is positively correlated with drift speed. Additionally cells with higher angular volatility are shown to have more rounded morphology. Future experiments taking advantage of discrete measurements of cytoskeletal arrangement and protein trafficking could be very useful in furthering the understanding of directed migration on haptotactic gradients.

**References:**

1 Singer and Kupfer. Annu. Rev. Cell Biol., 2, 337 (1986)

2 Smith et.al. *Langmuir*, 20, 8279 (2004)

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